09/598507
A11 #7

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=> s supercoil?
L1 18040 SUPERCOIL?

=> s hydrophobic
L2 236069 HYDROPHOBIC

=> s purif? or separat? or isolat?
L3 5628458 PURIF? OR SEPARAT? OR ISOLAT?

=> s l1 and l2 and l3
L4 35 L1 AND L2 AND L3

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5 19 DUP REM L4 (16 DUPLICATES REMOVED)

=> s purif?
L6 1781609 PURIF?

=> s separat?
L7 1730365 SEPARAT?

=> s isolat?
L8 3039314 ISOLAT?

=> s l1 and l2 and l6
L9 23 L1 AND L2 AND L6

=> s l1 and l2 and l7
L10 20 L1 AND L2 AND L7

=> s l1 and l2 and l8
L11 15 L1 AND L2 AND L8

=> s l9 or l10 or l11
L12 35 L9 OR L10 OR L11

=> dup rem l12
PROCESSING COMPLETED FOR L12
L13 19 DUP REM L12 (16 DUPLICATES REMOVED)

=> d l13 ibib kwic 1-19

L13 ANSWER 1 OF 19 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD DUPLICATE 1

ACCESSION NUMBER: 2001-390044 [41] WPIDS
DOC. NO. CPI: C2001-118874
TITLE: ***Isolating*** nucleic acid molecules from a cell comprises contacting a cell with a solution containing a biopolymer-degrading enzyme and a ***hydrophobic*** surfactant to yield a cell suspension.
DERWENT CLASS: A96 B04 D16
INVENTOR(S): BERNINGER, R W; GOFFE, A D; GOFFE, R A
PATENT ASSIGNEE(S): (GENE-N) GENESPAN CORP
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001042456	A2	20010614	(200141)*	EN	44

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001042456	A2	WO 2000-US42732	20001208

PRIORITY APPLN. INFO: US 2000-241638 20001019; US 1999-170185 19991210

TI ***Isolating*** nucleic acid molecules from a cell comprises contacting a cell with a solution containing a biopolymer-degrading enzyme and a ***hydrophobic*** surfactant to yield a cell suspension.

AB WO 200142456 UPAB: 20010724

NOVELTY - ***Isolating*** nucleic acid molecules from a cell comprises contacting a cell with a solution containing a biopolymer-degrading enzyme and then contacting the cell with a solution containing a ***hydrophobic*** surfactant to yield a cell suspension.

DETAILED DESCRIPTION - ***Isolating*** (M1) nucleic acid molecules from a cell comprises:

(a) contacting a cell with a solution comprising a biopolymer-degrading enzyme that is not a nuclease; and

(b) contacting the cell with a solution comprising a ***hydrophobic*** surfactant to yield a cell suspension comprising cell, biopolymer-degrading enzyme and ***hydrophobic*** surfactant.

The ***hydrophobic*** surfactant has a critical micelle concentration less than 3.0mM and its concentration in the cell suspension is at least 0.05% (v/v).

INDEPENDENT CLAIMS are also included for the following:

(I) ***Isolating*** nucleic acids (M2) from a cell comprising:

(a) suspending a cell in a solution comprising a carbohydrate degrading enzyme to form a cell suspension (I);

(b) adding to (I) an amount of ***hydrophobic*** surfactant at a concentration of at least 0.05% (v/v) and less than 3.0 mM and adding an amount of an. . . of neutralizing agent sufficient to adjust the pH of the solution to between pH 6.5 to pH 7.5;

(2) An ***isolated*** nucleic acid preparation with an A260/230 ratio of at least 2.0 and prepared by M1 or M2;

(3) An ***isolated*** DNA preparation comprising at least 80% ***supercoiled*** DNA; and

(4) ***Isolated*** plasmid DNA (I) that encodes and expresses a protein for 10, 15 or 20 days after induction into a mammalian. . . the same type of mammalian cell in vivo which is the same as (I) except that it is prepared by ***purification*** twice on a cesium chloride gradient instead of M1 or M2.

USE - M1 and M2 are useful for ***isolating*** nucleic acid from a cell (claimed).

ADVANTAGE - Yields ***isolated*** ***purified*** nucleic acid freer form contaminants such as bacterial endotoxins than prior art methods. The methods reduce the viscosity of cell. . .

TECH. . . UPTX: 20010724

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: In M1 the solution comprising the biopolymer degrading enzyme and the ***hydrophobic*** surfactant are the same or different solutions and the cell is contacted with the enzyme first then the surfactant or both at the same time. The concentration of ***hydrophobic*** surfactant the cell suspension is at least 0.1%, 0.15%, or 0.2% (v/v) and has a critical micelle concentration of 0.1mM, 0.5mM, 1.0mM or 2.0mM. The ***hydrophobic*** surfactant has a hydrophile lipophile balance number of less than 20 or 15 and has a solubility of less than 2g, 1.5g or 1g/100ml in water. The solution comprising a ***hydrophobic*** surfactant further comprises a non- ***hydrophobic*** surfactant with a critical micelle concentration greater than 3.0mM, preferably 5.0mM or 7.0mM, and the concentration is at least 0.4% (v/v), preferably at least 0.5 or 0.6% (v/v). The non- ***hydrophobic*** surfactant has a hydrophile lipophile balance number of greater than 20 or 30 and a solubility greater than 2 g/100ml water. Preferably the ***hydrophobic*** surfactant is in a concentration of 0.2%(v/v) and the non- ***hydrophobic*** surfactant is 0.4% (v/v). The cell is a eukaryote or a prokaryote. M1 further comprises the step of ***isolating*** nucleic acid having an A260/A230 ratio of at least 2.0, and is preferably DNA, more preferably plasmid DNA. In M2 the cell is contacted with the ***hydrophobic*** surfactant for

a period of 3 to 12 minutes before adding the neutralizing agent and the neutralizing agent is an acidic salt. M2 further comprises adding a non-***hydrophobic*** surfactant as in M1.

Preferred Enzyme: The biopolymer degrading enzyme is a carbohydrate degrading, protein degrading or lipid-degrading enzyme preferably a carbohydrate degrading enzyme more preferably alpha-amylase, beta-amylase,

amyloglucosidase, invertase, and glycopeptidase F.

Preferred Nucleic Acid: The ***isolated*** DNA preparation is preferably 90% or 95% ***supercoiled*** DNA.

TECHNOLOGY FOCUS - ORGANIC CHEMISTRY - The ***hydrophobic***

surfactant is polyoxoethylene sorbitan monolaureate, polyoxoethylene sorbitan monooleate, Triton X-100, Triton X-114, N-tetradecyl-N, N-dimethyl-3-ammonio-1-propanesulfonate, sodium dioctyl sulfosuccinate,

surfynol(RTM) 420, surfynol(RTM) 440, surfynol(RTM) 465, surfynol(RTM) 485

and TR-70.

The non-***hydrophobic*** surfactant is sodium dodecyl sulfate or CHAPS.

TT TT: ***ISOLATE*** NUCLEIC ACID MOLECULAR CELL COMPRISE CONTACT CELL

SOLUTION CONTAIN DEGRADE ENZYME ***HYDROPHOBIC*** SURFACTANT YIELD CELL SUSPENSION.

L13 ANSWER 2 OF 19 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD DUPLICATE 2

ACCESSION NUMBER: 2001-049925 [06] WPIDS
DOC. NO. CPI: C2001-013739
TITLE: ***Purifying*** plasmid DNA, useful in gene therapy, by selective retention of impurities, particularly endotoxin, on ***hydrophobic*** interaction resin.
DERWENT CLASS: A96 B04 D16
INVENTOR(S): RAMASUBRAMANYAN, N
PATENT ASSIGNEE(S): (BIOS-N) BIO SCI CONTRACT PRODN CORP
COUNTRY COUNT: 91
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000073318	A1	20001207	(200106)*	EN	58

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW

W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

AU 2000051655 A 20001218 (200118)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000073318	A1	WO 2000-US14527	20000526
AU 2000051655	A	AU 2000-51655	20000526

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2000051655	A Based on	WO 200073318

PRIORITY APPLN. INFO: US 1999-136772 19990528

TI ***Purifying*** plasmid DNA, useful in gene therapy, by selective retention of impurities, particularly endotoxin, on ***hydrophobic*** interaction resin.

AB WO 200073318 UPAB: 20010126

NOVELTY - ***Purification*** of plasmid DNA (I) from a mixture containing at least 1 host cell impurity (II) by adding enough salt to the mixture to allow selective binding of at least 1 (II) to a ***hydrophobic*** interaction medium (III), treating the resulting solution with (III) so that (II) binds to form a complex, and recovering unbound. . . detergents, glycols, hexamine cobalt, spermidine or poly(vinyl pyrrolidone).

INDEPENDENT CLAIMS are also included for the following:

(a) a method for ***separating*** ***supercoiled*** plasmid DNA (Ia) from a mixture containing relaxed plasmid DNA (Ib) and optionally at least 1 (II);

(b) a method for ***separation*** of endotoxin (IIa) from (I) by treatment with (III) to form a complex with (IIa);

(c) a method for enriching the amount of (Ia) relative to (Ib); and

(d) a method for ***separating*** lipopolysaccharide (LPS) from a DNA-containing composition.

USE - The method is especially used to remove endotoxin (lipopolysaccharide), but also RNA,. . . has a much higher selectivity for lipopolysaccharide and lipoproteins than for DNA over a wide pH range and also allows ***separation*** of ***supercoiled*** and relaxed plasmids. Typically the impurity contents are reduced to 1-300 endotoxin units/mg DNA, to below 0.1wt.% for protein and. . . TECH. . . about 2 M, and the solution has a pH of 6.8-7.4, particularly 7.4. (III) is a chromatography support with pendant ***hydrophobic*** groups, preferably 3-10C alkyl. Most preferably it has a methacrylate-ethylene glycol copolymer or crosslinked agarose backbone, and is especially in. .

TT TT: ***PURIFICATION*** PLASMID DNA USEFUL GENE THERAPEUTIC SELECT

RETAIN IMPURE ENDOTOXIN ***HYDROPHOBIC*** INTERACT RESIN.

L13 ANSWER 3 OF 19 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 3

ACCESSION NUMBER: 2000177966 EMBASE
TITLE: ***Purification*** of a cystic fibrosis plasmid vector for gene therapy using ***hydrophobic*** interaction chromatography.
AUTHOR: Diogo M.M.; Queiroz J.A.; Monteiro G.A.; Martins S.A.M.; Ferreira G.N.M.; Prazeres D.M.F.
CORPORATE SOURCE: D.M.F. Prazeres, Ctro. Engenharia Biologica e Quimica, Instituto Superior Tecnico, Av. Rovisco Pais, 1000 Lisboa, Portugal. prazeres@alfa.ist.utl.pt
SOURCE: Biotechnology and Bioengineering, (5 Jun 2000) 68/5 (576-583).
Refs: 18
ISSN: 0006-3592 CODEN: BIBIAU
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

TI ***Purification*** of a cystic fibrosis plasmid vector for gene therapy using ***hydrophobic*** interaction chromatography.

AB . . . the ability to produce large amounts of plasmid DNA according to defined specifications. A new method is described for the ***purification*** of a cystic fibrosis plasmid vector (pCF1- CFTR) of clinical grade, which includes an ammonium sulfate precipitation followed by ***hydrophobic*** interaction chromatography (HIC) using a Sepharose gel derivatized with 1,4-butanedioldiglycidylether. The use of HIC took advantage of the more ***hydrophobic*** character of single-stranded nucleic acid impurities as compared with double-stranded plasmid DNA. RNA, denatured genomic and plasmid DNAs, with large stretches of single strands, and lipopolysaccharides (LPS) that are more ***hydrophobic*** than super-coiled plasmid, were retained and ***separated*** from non-binding plasmid DNA in a 14-cm HIC column.

Anion-exchange HPLC analysis proved that >70% of the loaded plasmid was.

. .

CT Medical Descriptors:
*cystic fibrosis: TH, therapy
*plasmid vector
*gene therapy

*chromatography
hydrophobicity
derivatization
anion exchange chromatography
high performance liquid chromatography
precipitation
polyacrylamide gel electrophoresis
DNA supercoiling
nonhuman
article
*plasmid DNA
sepharose
1,4 butanediol
single stranded DNA
lipopolysaccharide
ammonium acetate

L13 ANSWER 4 OF 19 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:327378 HCAPLUS
TITLE: Novel chromatographic resins for the ***purification*** of ***supercoiled*** plasmid DNA.
AUTHOR(S): O'Donnell, J. Kevin; Fisher, Jon R.; Picciotti, Robert A.; Yamasaki, Oscar
CORPORATE SOURCE: Technical Service, TosoHaas, Montgomeryville, PA, 18936, USA
SOURCE: Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000 (2000), BIOT-153. American Chemical Society: Washington, D. C. CODEN: 69CLAC
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
TI Novel chromatographic resins for the ***purification*** of ***supercoiled*** plasmid DNA.
AB Gene Therapy continues to grow as a new discipline in the pharmaceutical industry. Of utmost importance is the ***purifn*** . of the vector whether it be viral or nonviral in nature. The marriage of chromatog. techniques with viral and DNA ***purifn*** . at scales large enough to support clin. trials has been rather arduous. Recently however, the application of ***Hydrophobic*** Interaction Chromatog. for the ***purifn*** . of plasmids was reported. Using a very ***hydrophobic*** chromatog. resin, Toyopearl Hexyl-650C, the sepn. of ***supercoiled*** from open circular was accomplished with std. lab. conditions. This resin has a hexyl ligand covalently attached to a very hydrophilic polymethacrylate backbone. The pore size is nominally 1000.ANG. and the particle size is 50-150.mu.m. New chromatog. resins contg. even larger pores were synthesized to accommodate the size of gene therapy vectors. These resins include both ion exchange and ***hydrophobic*** interaction modes. Investigating the effect of pore size and particle size will help to optimize the resoln. and dynamic binding capacity of ***supercoiled*** plasmids on chromatog. resins. Studies are currently underway to quickly and efficiently characterize the sepn. of bacterial host proteins from the desired ***supercoiled*** plasmid using techniques that do not require the use of org. solvents or added proteins.

L13 ANSWER 5 OF 19 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:688475 HCAPLUS
DOCUMENT NUMBER: 132:61133
TITLE: ***Separation*** and Analysis of Plasmid Denatured Forms Using ***Hydrophobic*** Interaction Chromatography
AUTHOR(S): Diogo, M. M.; Queiroz, J. A.; Monteiro, G. A.; Prazeres, D. M. F.
CORPORATE SOURCE: Centro de Engenharia Biologica e Quimica, Instituto Superior Tecnico, Lisbon, 1000, Port.
SOURCE: Anal. Biochem. (1999), 275(1), 122-124 CODEN: ANBCA2; ISSN: 0003-2697
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 5
REFERENCE(S): (1) Birnboim, H; Nucleic Acids Res 1979, V7, P1513 HCAPLUS
(2) Prazeres, D; J Chromatogr A 1998, V806, P31 HCAPLUS
(3) Queiroz, J; J Chromatogr A 1995, V707, P137 HCAPLUS
(4) Sayers, J; Anal Biochem 1996, V241, P186 HCAPLUS
(5) Sundberg, L; J Chromatogr 1974, V90, P87 HCAPLUS
TI ***Separation*** and Analysis of Plasmid Denatured Forms Using ***Hydrophobic*** Interaction Chromatography
AB This work explores the possibility of using a ***hydrophobic*** interaction chromatog. (HIC) support to sep. ***supercoiled*** plasmids from denatured forms, by taking advantage of their different surface hydrophobicity. The ***hydrophobic*** gel used in this work was prepd. by covalent immobilization of 1,4-butanediol diglycidyl ether on Sepharose CL-6B (Pharmacia). The ***hydrophobic*** interaction between this support and lipases was previously reported. Expts. were carried out in a 16 x 150-mm column packed with this gel and equilibrated with 10 mM Tris, pH 8, with 1.5 M (NH4)2SO4 at a flow rate of 60 mL/h. The absorbance was monitored at 254 nm. The plasmid used in the expts. was produced by fermn. of E. coli DH5.alpha. competent cells transformed with the 8.5-kb pCF1-CFTR plasmid (Genzyme Corp.). Growth was carried out overnight in LB medium (30,ug/mL kanamycin), in 100-mL shake-flasks at 37.degree. and 250 rpm. This work shows that HIC can be used for the sepn. of plasmid variants. The technique can play an important role in the preparative ***purifn*** . of super-coiled plasmids for gene therapy and DNA vaccination. In fact, the HIC support studied was capable of removing denatured plasmid variants that are usually produced with the widespread method of alk. lysis of plasmid ***isolation*** . This is very difficult to achieve using other chromatog. processes. Another important application could be in the monitoring and quality control of ***purified*** plasmids. Ongoing work indicates also an ability of the HIC support to sep. RNA and genomic DNA from plasmids. (c) 1999 Academic Press.
ST ***hydrophobic*** interaction chromatog plasmid ***purifn***
IT Plasmids
(pCF1-CFTR; sepn. and anal. of plasmid denatured forms using ***hydrophobic*** interaction chromatog.)
IT Escherichia coli
Fermentation
Gene therapy
Hydrophobic interaction chromatography
Immobilization, biochemical
Liquid chromatographic stationary phases
Quality control
(sepn. and anal. of plasmid denatured forms using ***hydrophobic*** interaction chromatog.)
IT Immunization
(vaccination; sepn. and anal. of plasmid denatured forms using ***hydrophobic*** interaction chromatog.)
IT 2425-79-8, 1,4-Butanediol diglycidyl ether 62610-50-8, Sepharose CL 6B
RL: ARU (Analytical role, unclassified); RCT (Reactant); ANST (Analytical study)
(sepn. and anal. of plasmid denatured forms using ***hydrophobic*** interaction chromatog.)

L13 ANSWER 6 OF 19 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1999156202 EMBASE
TITLE: Capillary gel electrophoresis of nucleic acids in pluronic F127 copolymer liquid crystals.
AUTHOR: Rill R.L.; Liu Y.; Ramey B.A.; Van Winkle D.H.; Locke B.R.
CORPORATE SOURCE: R.L. Rill, Department of Chemistry, Institute of Molecular Biophysics, Florida State University, Tallahassee, FL, United States
SOURCE: Chromatographia, (1999) 49/SUPPL. 1 (S65-S71). Refs: 20 ISSN: 0009-5893 CODEN: CHRGB7
COUNTRY: Germany

DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB . . . approximate formula (EO)106 (PO)70 (EO)106. Polymer chains aggregate into spherical micelles in aqueous solutions, with poly(propylene oxide) chains creating a ***hydrophobic*** core surrounded by brushes of hydrated poly(ethylene oxide) tails. Crowding at high concentrations promotes ordering of micelles. Solutions in the. . . viscosity liquids that are easily loaded into capillaries. This article reviews applications of Pluronic F127 media for capillary gel electrophoresis ***separations*** of nucleic acids of several types including oligonucleotides, double stranded DNA fragments, and ***supercoiled*** plasmid DNAs.

L13 ANSWER 7 OF 19 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 4
ACCESSION NUMBER: 1998278790 EMBASE
TITLE: Pluronic copolymer liquid crystals: Unique, replaceable media for capillary gel electrophoresis.
AUTHOR: Rill R.L.; Liu Y.; Van Winkle D.H.; Locke B.R.
CORPORATE SOURCE: R.L. Rill, Department of Chemistry, Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306-4390, United States
SOURCE: Journal of Chromatography A, (1998) 817/1-2 (287-295). Refs: 37
ISSN: 0021-9673 CODEN: JCRAEY
PUBLISHER IDENT.: S 0021-9673(98)00522-6
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB . . . oxide) [(EO)(x)] and poly(propylene oxide) [(PO)(y)] with the general formula (EO)(x)(PO)(y)(EO)(x). Large micelles form in aqueous solutions in which central, ***hydrophobic*** cores of (PO)(y) segments are surrounded by “brushes” of hydrated (EO)(x) tails. Solutions of Pluronic F127 (BASF Performance Chemicals) in. . . of spherical micelles with diameters of 17-18 nm which pack with local cubic symmetry. CGE in Pluronic F127 liquid crystals ***separates*** species within several chemical classes as varied as nucleoside monophosphates and organic dyes, oligonucleotides of 4-60 nucleotides, DNA fragments of 50-3000 base pairs (bp), and ***supercoiled*** plasmid DNAs of 2000-10 000 bp. Mechanisms of molecular sieving in polymer liquid crystals must differ in fundamental ways from ***separations*** in random polymer gels because molecules move around uncrosslinked obstacles that are larger than the smallest dimensions of typical analytes.. . . (EO)(x) strands of micelle brushes, or through brushtips and interstitial spaces between micelles. Small molecules such as nucleotides appear to ***separate*** by a different mechanism involving partitioning between hydrophilic and ***hydrophobic*** environments. This process is termed “ ***hydrophobic*** interaction electrophoresis”. The unique structures of Pluronic copolymers and their liquid crystalline phases provide new challenges and opportunities in ***separations*** science. Copyright (C) 1998 Elsevier Science B.V.

L13 ANSWER 8 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1993:387799 BIOSIS
DOCUMENT NUMBER: PREV199396063099
TITLE: ***Purification*** and cDNA cloning of bovine liver 5'-nucleotidase, a GPI-anchored protein, and its expression in COS cells.
AUTHOR(S): Suzuki, Kensuke; Furukawa, Yoko; Tamura, Hiro-Omi; Ejiri, Noritaka; Suematsu, Hiroyuki; Taguchi, Ryo; Nakamura, Shin; Suzuki, Yoshiaki; Ikezawa, Hiroh (1)
CORPORATE SOURCE: (1) Dep. Microbial Chem., Fac. Pharm. Sci., Nagoya City Univ., Tanabe-dori, Mizuho-ku, Nagoya 467 Japan
SOURCE: Journal of Biochemistry (Tokyo), (1993) Vol. 113, No. 5, pp. 607-613.
ISSN: 0021-924X.
DOCUMENT TYPE: Article
LANGUAGE: English
TI ***Purification*** and cDNA cloning of bovine liver 5'-nucleotidase, a GPI-anchored protein, and its expression in COS cells.
AB. . . 3.1.3.5), was released from the membrane of bovine liver by use of phosphatidylinositol-specific phospholipase C (PI-PLC) of Bacillus thuringiensis and ***purified*** by several column chormatographies to a homogeneous state. The ***purified*** protein has an apparent molecular mass of 61 kDa, as estimated by SDS-polyacrylamide gel electrophoresis. From the partial amino acid. . . peptide, mixed oligonucleotides were synthesized and used to screen a lambda-gt11 liver cDNA library, and one positive clone, pE1, was ***isolated*** . Since the insert of the clone lacked the NH-2-terminal coding region, another lambda-gt11 liver cDNA library was screened by using. . . reading frame that encodes a 574-residue polypeptide with a calculated mass of 63,084 Da. The predicted structure showed two highly ***hydrophobic*** stretches at both ends of the protein, like those of rat and human 5'-nucleotidases. The NH-2-terminal 26 residues comprise a signal peptide and the COOH-terminal ***hydrophobic*** stretch may serve as a signal for the posttranslational GPI modification. An expression vector of the cDNA, pSVNT, was constructed. . . times higher than the pSVL-transfected control activity. PI-PLC released 45% of the transiently expressed 5'-nucleotidase activity, indicating that the cDNA ***isolated*** here encodes this enzyme expressed as a GPI-anchored protein.
IT Miscellaneous Descriptors
DNA ***SUPERCOILING*** ; REPLICATION; TRANSCRIPTION

L13 ANSWER 9 OF 19 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 92337651 EMBASE
DOCUMENT NUMBER: 1992337651
TITLE: Preparation of DNA topoisomers by RP-18 high-performance liquid chromatography.
AUTHOR: Kapp U.; Langowski J.
CORPORATE SOURCE: EMBL, Grenoble Outstation, P.O. Box 156X,F-38042 Grenoble Cedex, France
SOURCE: Analytical Biochemistry, (1992) 206/2 (293-299).
ISSN: 0003-2697 CODEN: ANBCA2
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A method for the ***separation*** of superhelical DNA on the basis of superhelical density by reverse-phase HPLC on RP-18 columns is described.
The technique can. . . be used to prepare superhelical DNA in milligram amounts and narrow topoisomer distributions in 0.1 mg amounts. We show example ***separations*** of the plasmids pUC18 (2687 bp) and .pi.AN13 (895 bp). While the best ***separation*** for pUC18 yields topoisomer distributions of two or three major components, the small plasmid can be ***separated*** into single topoisomer fractions. The basis of the ***separation*** is probably an interaction of partially opened bases with the ***hydrophobic*** column matrix. This hypothesis is supported by the elution behavior of DNA fragments on this column: DNA fragments with sticky. . .
CT Medical Descriptors:
*dna determination
*reversed phase high performance liquid chromatography
animal cell
article
cell separation
column chromatography
dna structure
dna supercoiling
hydrophobicity
intercalation complex
ligand binding
nonhuman

plasmid
priority journal
*dna topoisomerase: EC, endogenous compound

L13 ANSWER 10 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 5
ACCESSION NUMBER: 1992:347184 BIOSIS
DOCUMENT NUMBER: BA94:39409
TITLE: DNA-INTERCALATORS INTERACTION WITH DNA AND OTHER CELLULAR
COMPONENTS AND APPLICATION IN EXPERIMENTAL BIOLOGY.
AUTHOR(S): FADDEEVA M D; BELYAEV T N
CORPORATE SOURCE: INST. CYTOL., ACAD. SCI. RUSS., ST. PETERSBURG, RUSS.
SOURCE: TSITOLOGIYA, (1991) 33 (10), 3-31.
CODEN: TSITAQ. ISSN: 0041-3771.
FILE SEGMENT: BA; OLD
LANGUAGE: Russian
AB. . . Besides, other physico-chemical criteria of DNA-intercalation are as following: the increase in the contour length of duplex DNA; unwinding of ***supercoils*** from natural ***supercoiled*** covalently-closed duplex DNA; the increase in Tm of DNA in the complexes with ligands. The changes of spectral properties of. . . fluorescent assays of activities of various enzymes involved in nucleic acid metabolism; chromosome identification according to their fluorescent banding patterns; ***separation*** of nucleic acid topological forms, and many other methods. The inhibition of reactions of DNA replication, transcription, topoisomerization and of enzymatic degradation by DNA-intercalators represents an important consequence of DNA structure modification due to intercalation. Besides, as ***hydrophobic*** cations DNA-intercalators uncouple the oxidative phosphorylation in mammalian cell mitochondria. There are some other protein and phospholipid targets for DNA-intercalators. . .

L13 ANSWER 11 OF 19 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 6
ACCESSION NUMBER: 89196552 EMBASE
DOCUMENT NUMBER: 1989196552
TITLE: Of matrices and men.
AUTHOR: Righetti P.G.
CORPORATE SOURCE: Faculty of Pharmacy, Department of Biomedical Sciences and Technologies, University of Milan, Milan 20133, Italy
SOURCE: Journal of Biochemical and Biophysical Methods, (1989) 19/1 (1-20).
ISSN: 0165-022X CODEN: JBBMDG
COUNTRY: Netherlands
DOCUMENT TYPE: Journal
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB . . . analyses in clinical chemistry labs. The properties of agarose are discussed, in particular its capacity of forming large-pore structures via ***supercoiling*** , i.e. formation of suprafibers with average radii of .apprx.20-25 nm. Several modified agaroses are reviewed, in particular the SeaPlaque, SeaPrep,. . . NuSieve, NuFix, Seakem and Isogel brands and a composite agarose-polyacrylamide matrix, quite popular in the seventies for DNA and RNA ***separations*** . The field of polyacrylamide gels seems to be bursting, with the large number of crosslinkers described, imparting special properties to. . . first time. The review culminates with a glimpse at a new generation of amphiphatic matrices, such as HydroLink and 'shielded ***hydrophobic*** phase' gels, which appear to be the latest developments in the fields of electrophoresis and chromatography, respectively.

L13 ANSWER 12 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 7
ACCESSION NUMBER: 1989:128967 BIOSIS
DOCUMENT NUMBER: BA87:63620
TITLE: REVERSE GYRASE OF SULFOLOBUS ***PURIFICATION*** TO HOMOGENEITY AND CHARACTERIZATION.
AUTHOR(S): NADAL M; JAXEL C; PORTEMER C; FORTERRE P; MIRAMBEAU G; DUGUET M
CORPORATE SOURCE: LAB. ENZYMOL. ACIDES NUCL., UA 554 CNRS, UNIV. PIERRE ET MARIE CURE, 96 BLVD. RASPAIL, 75006 PARIS, FR.
SOURCE: BIOCHEMISTRY, (1988) 27 (26), 9102-9108.
CODEN: BICHAW. ISSN: 0006-2960.
FILE SEGMENT: BA; OLD
LANGUAGE: English
TI REVERSE GYRASE OF SULFOLOBUS ***PURIFICATION*** TO HOMOGENEITY AND CHARACTERIZATION.
AB By using ***hydrophobic*** interaction as the first chromatographic stage, we ***purified*** to homogeneity reverse gyrase, an ATP-dependent DNA topoisomerase I, ***isolated*** from the thermoacidophilic archaebacterium Sulfolobus acidocaldarius. This procedure allowed quick and complete ***separation*** of reverse gyrase from nucleases and DNA binding proteins present in Sulfolobus. The final product was revealed, by SDS-PAGE, as. . . acid composition was determined. Western blotting experiments with antibodies raised against reverse gyrase indicate that no proteolysis occurred during the ***purification*** course. Gel filtration and sedimentation data gave a Stokes radius of 42 .ANG. and a sedimentation coefficient of 5.7 S,. . . which was confirmed by electron microscopy. Finally, pure reverse gyrase in a monomeric state was still able to promote positive ***supercoiling*** of the DNA.

L13 ANSWER 13 OF 19 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1988:218996 HCAPLUS
DOCUMENT NUMBER: 108:218996
TITLE: DNA strand scission by naturally occurring 5-alkylresorcinols
AUTHOR(S): Scannell, Ralph T.; Barr, John R.; Murty, V. S.; Reddy, K. Sambi; Hecht, Sidney M.
CORPORATE SOURCE: Dep. Chem., Univ. Virginia, Charlottesville, VA, 22901, USA
SOURCE: J. Am. Chem. Soc. (1988), 110(11), 3650-1
CODEN: JACSAT; ISSN: 0002-7863
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Three 5-alkylresorcinol derivs. capable of mediating strand scission of ***supercoiled*** covalently closed circular DNA in the presence of Cu(II) were ***isolated*** from a dichloromethane ext. of Hakea trifurcata by bioassay guided fractionation. Structure detn. was accomplished by mass spectrometry, IR and UV spectroscopy, as well as 1H, 13C, and 2-dimensional homonuclear correlation NMR spectroscopy, which indicated the compds. to be 1,3-dihydroxy-5-tridecylbenzene, 1,3-dihydroxy-5-pentadec-cis-8'-enylbenzene and 1,3-dihydroxy-5-(14'-(3",5"-dihydroxyphenyl)tetradec-cis-6'-enyl)benzene (I). The structures assigned to the former 2 were verified by total synthesis. A preliminary mechanistic study suggested that DNA strand scission by these agents may involve an obligatory oxygenation of the benzene ring. The oxygenated derivs. could then bind and reduce Cu(II) in the presence of O2, which would be anticipated to produce O free radicals. The efficiency of DNA cleavage for these 5-alkylresorcinols was proportional to the length of the 5-alkyl substituent, suggesting that there may be an assocn. of such lipophilic groups with the relatively ***hydrophobic*** interior of the DNA helix.
IT Hakea trifurcata
(alkylresorcinols of, ***isolation*** and structure detn. of and DNA strand scission by)
IT 5259-01-8 22910-86-7 52483-24-6
RL: BIOL (Biological study)
(of Hakea trifurcata, ***isolation*** and structure detn. of and DNA strand scission by)

L13 ANSWER 14 OF 19 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1986:511825 HCAPLUS
DOCUMENT NUMBER: 105:111825
TITLE: ***Isolation*** and characterization of norfloxacin-resistant mutants of Escherichia coli K-12
AUTHOR(S): Hirai, Keiji; Aoyama, Hiroshi; Suzue, Seigo; Irikura,

Tsutomu; Iyobe, Shizuko; Mitsuhashi, Susumu
CORPORATE SOURCE: Cent. Res. Lab., Kyorin Pharm. Co. Ltd., Tochigi,
Japan
SOURCE: Antimicrob. Agents Chemother. (1986), 30(2), 248-53
CODEN: AMACCQ; ISSN: 0066-4804
DOCUMENT TYPE: Journal
LANGUAGE: English
TI ***Isolation*** and characterization of norfloxacin-resistant mutants of Escherichia coli K-12
AB Spontaneous mutants were ***isolated*** from E. coli K-12 with low-level resistance to norfloxacin. These mutants were classified on the basis of their properties: (i) NorA appeared to result from mutation in the gyrA locus for the A subunit of DNA gyrase; (ii) NorB showed low-level
resistance to quinolones and other antimicrobial agents (e.g., cefoxitin, chloramphenicol, and tetracycline), and the norB gene was considered to map at .apprx.34 min on the E. coli K-12 chromosome; (iii) NorC was less susceptible to norfloxacin and ciprofloxacin but was hypersusceptible to ***hydrophobic*** quinolones such as nalidixic acid and rosoxacin, ***hydrophobic*** antibiotics, dyes, and detergents. Susceptibility to bacteriophages and the hydrophobicity of the NorC cell surface also differed from that of the parent strain. The norC gene was located near the lac locus at 8 min on the E. coli K-12 chromosome. Both NorB and NorC
mutants had a lower rate of norfloxacin uptake; the NorB mutant was altered in OmpF porin and the NorC mutant was altered in both OmpF porin
and apparently in the lipopolysaccharide structure of the outer membrane.
IT Escherichia coli
(norfloxacin-resistant mutants of, ***isolation*** and characterization of)
IT Enzymes
RL: BIOL (Biological study)
(DNA- ***supercoiling*** , gene for, of Escherichia coli, norfloxacin resistance in relation to)
IT 70458-96-7
RL: BIOL (Biological study)
(Escherichia coli mutants resistant to, ***isolation*** and characterization of)

L13 ANSWER 15 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS
DUPLICATE 8
ACCESSION NUMBER: 1986:279543 BIOSIS
DOCUMENT NUMBER: BA82:23406
TITLE: ANALYSIS AND ***PURIFICATION*** OF PLASMID DNA BY
REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY.
AUTHOR(S): COLOTE S; FERRAZ C; LIAUTARD J P
CORPORATE SOURCE: UNITE U. 249 INSERM, INST. DE BIOL., BLVD. HENRI IV, 34000
MONTPELLIER.
SOURCE: ANAL BIOCHEM, (1986) 154 (1), 15-20.
CODEN: ANBCA2. ISSN: 0003-2697.
FILE SEGMENT: BA; OLD
LANGUAGE: English
TI ANALYSIS AND ***PURIFICATION*** OF PLASMID DNA BY REVERSED-PHASE
HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY.
AB Large nucleic acids can be ***separated*** by reversed-phase high-performance liquid chromatography. Under our experimental conditions,
the retention time depends not on the chain length but rather on the base composition and the secondary structure of the molecule. Because of the torsional strain caused by the ***supercoiling*** of the plasmid, more of its bases are accessible for interaction with the ***hydrophobic*** stationary phase. This increases the retention time of the ***supercoiled*** DNA compared to the relaxed or linear DNA. We have
exploited these properties to analyze the quality of plasmid preparations.. . . The method is more sensitive to contaminants than common electrophoretic techniques. Furthermore, we describe a convenient
and rapid procedure for ***purifying*** plasmid DNA. The highly pure plasmid is biologically more active for most of the enzymatic reactions commonly used in genetic. . .

L13 ANSWER 16 OF 19 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 85134876 EMBASE
DOCUMENT NUMBER: 1985134876
TITLE: The involvement of nucleosomes in giemsa staining of chromosomes. A new hypothesis on the banding mechanism.
AUTHOR: Van Duijn P.; Van Prooijen-Knegt A.C.; Van Der Ploeg M.
CORPORATE SOURCE: Department of Histochemistry and Cytochemistry, Medical
Faculty, Sylvius Laboratories University of Leiden, NL-2333 AL Leiden, Netherlands
SOURCE: Histochemistry, (1985) 82/4 (363-376).
CODEN: HCMYAL
COUNTRY: Germany
DOCUMENT TYPE: Journal
FILE SEGMENT: 001 Anatomy, Anthropology, Embryology and Histology
022 Human Genetics
005 General Pathology and Pathological Anatomy
LANGUAGE: English
AB . . . of chromosomes. Giemsa staining as well as the concomitant swelling can be explained as an insertion of the triple charged ***hydrophobic*** dye complex between the negatively-charged ***supercoiled*** helical DNA and the denatured histone cores of the nucleosomes still present in the fixed chromosomes. New cytochemical data
and. . . this hypothesis. Chromosomes are stained by the Giemsa procedure in a purple (magenta) colour. Giemsa staining of DNA and histone
(***isolated*** or in a simple mixture) in model experiments results in different colours, indicating that a higher order configuration of these. . . transferred to aqueous buffer solutions. During homogeneous Giemsa staining reswelling of the unpretreated chromosome is explained by
insertion of the ***hydrophobic*** Giemsa complex between the ***hydrophobic*** nucleosome cores and the superhelix DNA. Selective
Giemsa staining of the AT-enriched bands after saline pretreatment is thought to be. . . as well as the selective band staining after pretreatment of the chromosomes primarily to differences in base composition of the ***supercoiled*** DNA helices present in bands and
interbands. Such base sequence differences are known to lead to differences in internal tension in ***supercoiled*** DNA helices as present in the nucleosomes as well as to differences in binding strength to the polar parts of. . .

L13 ANSWER 17 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS
DUPLICATE 9
ACCESSION NUMBER: 1982:154571 BIOSIS
DOCUMENT NUMBER: BA73:14555
TITLE: MECHANISM OF REPLICATION OF PHI-X-174 17. ***PURIFICATION*** AND PARTIAL CHARACTERIZATION OF THE
GENE A AND A ASTERISK PROTEINS.
AUTHOR(S): DUBEAU L; HOURS C; DENHARDT D T
CORPORATE SOURCE: CANCER RESEARCH LABORATORY, UNIVERSITY OF WESTERN ONTARIO,
LONDON, ONT., CANADA N6A 5B7.
SOURCE: CAN J BIOCHEM, (1981) 59 (2), 106-115.
CODEN: CJBIAE. ISSN: 0008-4018.
FILE SEGMENT: BA; OLD
LANGUAGE: English
TI MECHANISM OF REPLICATION OF PHI-X-174 17. ***PURIFICATION*** AND
PARTIAL CHARACTERIZATION OF THE GENE A AND A ASTERISK PROTEINS.
AB A simple method for ***purifying*** the gene A plus A* proteins of bacteriophage .vphi.X174 is presented. The proteins are relatively ***hydrophobic*** and precipitate from aqueous solutions at low ionic strength. The gene A plus A* protein preparation stimulated .vphi.X174 DNA
replication in vitro and nicked ***supercoiled*** .vphi.X174 replicative form I to produce unit-length linear and circular strands of DNA. The kinetics of the nicking reaction were. . .

L13 ANSWER 18 OF 19 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1977:208909 BIOSIS
DOCUMENT NUMBER: BA64:31273
TITLE: DNA BINDING SEGMENTS OF 4 HISTONE SEQUENCES IDENTIFIED IN TRYPSIN EC-3.4.21.4 TREATED H-1 DEPLETED CHROMATIN.
AUTHOR(S): KATO Y; IWAI K
SOURCE: J BIOCHEM (TOKYO), (1977) 81 (3), 621-630. CODEN: JOBIAO. ISSN: 0021-924X.
FILE SEGMENT: BA; OLD
LANGUAGE: Unavailable
AB. . . After 11-13 h in duplicate digestions, a chromatin fraction was precipitated in 80% (vol/vol) ethanol and 4 mM NaCl to ***separate*** the material from the peptides released. The trypsin-treated chromatin showed a thermal denaturation profile different from that of H1-depleted chromatin. . . (wt/wt) of the histones present in H1-depleted chromatin, and showed increased contents of basic amino acids and decreased contents of ***hydrophobic*** ones. These peptides were fractionated by repeated chromatography and analyzed for their total and N-terminal amino acids. About 50 peptide. . . N-terminal regions and secondarily at their C-terminals, each interacting with the middle regions and thus constraining the DNA into a ***supercoil*** .

L13 ANSWER 19 OF 19 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 74075173 EMBASE
DOCUMENT NUMBER: 1974075173
TITLE: Analysis of the primary structure of collagen for the origins of molecular packing.
AUTHOR: Hulmes D.J.S.; Miller A.; Parry D.A.D.; et al.
CORPORATE SOURCE: Lab. Molec. Biophys., Dept. Zool., Univ. Oxford, United Kingdom
SOURCE: Journal of Molecular Biology, (1973) 79/1 (137-148). CODEN: JMOBAK
DOCUMENT TYPE: Journal
FILE SEGMENT: 029 Clinical Biochemistry
031 Arthritis and Rheumatism
LANGUAGE: English
AB . . . range of .+-.2 to 3 residues. It was found that interactions between amino acids of opposite charge and between large ***hydrophobic*** amino acids in the overlapping region between two chains are maximal when the chains are staggered by 0D, 1D, 2D,. . . .+-. 1 residues. The residue repeat derived from this value is 2.86 .+-. 0.02 .ANG.. The existence of a D ***separation*** between interacting residues was shown to be reflected in the actual distribution of large ***hydrophobic*** amino acids. Surprisingly, the distribution approximates the pattern (2D/11)5(D/11) repeated over 4.4D intervals. The regularity may arise from structural constraints imposed by ***supercoiling*** . The distribution of charged residues is less regular and does not show a well defined periodicity. However, positively charged residues. . .

=> s endotoxin or lps

L14 157356 ENDOTOXIN OR LPS

=> d his

(FILE 'HOME' ENTERED AT 09:45:16 ON 16 SEP 2001)

FILE 'BIOSIS, EMBASE, MEDLINE, WPIDS, HCAPLUS' ENTERED AT 09:45:30 ON 16 SEP 2001
L1 18040 S SUPERCOIL?
L2 236069 S HYDROPHOBIC
L3 5628458 S PURIF? OR SEPARAT? OR ISOLAT?
L4 35 S L1 AND L2 AND L3
L5 19 DUP REM L4 (16 DUPLICATES REMOVED)
L6 1781609 S PURIF?
L7 1730365 S SEPARAT?
L8 3039314 S ISOLAT?
L9 23 S L1 AND L2 AND L6
L10 20 S L1 AND L2 AND L7
L11 15 S L1 AND L2 AND L8
L12 35 S L9 OR L10 OR L11
L13 19 DUP REM L12 (16 DUPLICATES REMOVED)
L14 157356 S ENDOTOXIN OR LPS

=> s reverse(w)phase(w)chromatography
L15 2210 REVERSE(W) PHASE(W) CHROMATOGRAPHY

=> s l1 and l15
L16 0 L1 AND L15

=> s plasmid?
L17 315171 PLASMID?

=> s l5 and l17
L18 9 L5 AND L17

=> dup rem l18
PROCESSING COMPLETED FOR L18
L19 9 DUP REM L18 (0 DUPLICATES REMOVED)

=> d l19 ibib kwic 1-9

L19 ANSWER 1 OF 9 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2001-390044 [41] WPIDS
DOC. NO. CPI: C2001-118874
TITLE: ***Isolating*** nucleic acid molecules from a cell comprises contacting a cell with a solution containing a biopolymer-degrading enzyme and a ***hydrophobic*** surfactant to yield a cell suspension.
DERWENT CLASS: A96 B04 D16
INVENTOR(S): BERNINGER, R W; GOFFE, A D; GOFFE, R A
PATENT ASSIGNEE(S): (GENE-N) GENESPAN CORP
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001042456	A2	20010614	(200141)*	EN	44

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001042456	A2	WO 2000-US42732	20001208

PRIORITY APPLN. INFO: US 2000-241638 20001019; US 1999-170185 19991210
TI ***Isolating*** nucleic acid molecules from a cell comprises contacting a cell with a solution containing a biopolymer-degrading enzyme and a ***hydrophobic*** surfactant to yield a cell suspension.
AB WO 200142456 UPAB: 20010724
NOVELTY - ***Isolating*** nucleic acid molecules from a cell comprises contacting a cell with a solution containing a biopolymer-degrading enzyme and then contacting the cell with a solution containing a ***hydrophobic*** surfactant to yield a cell suspension.
DETAILED DESCRIPTION - ***Isolating*** (M1) nucleic acid molecules from a cell comprises:
(a) contacting a cell with a solution comprising a biopolymer-degrading enzyme that is not a nuclease; and
(b) contacting the cell with a solution comprising a ***hydrophobic*** surfactant to yield a cell suspension comprising cell, biopolymer-degrading enzyme and ***hydrophobic*** surfactant.

The ***hydrophobic*** surfactant has a critical micelle concentration less than 3.0mM and its concentration in the cell suspension is at least 0.05% (v/v).

INDEPENDENT CLAIMS are also included for the following:

(1) ***Isolating*** nucleic acids (M2) from a cell comprising:

(a) suspending a cell in a solution comprising a carbohydrate degrading enzyme to form a cell suspension (I);

(b) adding to (I) an amount of ***hydrophobic*** surfactant at a concentration of at least 0.05% (v/v) and less than 3.0 mM and adding an amount of an. . . of neutralizing agent sufficient to adjust the pH of the solution to between pH 6.5 to pH 7.5;

(2) An ***isolated*** nucleic acid preparation with an A260/230 ratio of at least 2.0 and prepared by M1 or M2;

(3) An ***isolated*** DNA preparation comprising at least 80% ***supercoiled*** DNA; and

(4) ***Isolated*** ***plasmid*** DNA (I) that encodes and expresses a protein for 10, 15 or 20 days after induction into a mammalian cell. . . 50% after reaching the peak protein expression level, the time period being two times longer than expression of a reference ***plasmid*** DNA in the same type of mammalian cell in vivo which is the same as (I) except that it is prepared by ***purification*** twice on a cesium chloride gradient instead of M1 or M2.

USE - M1 and M2 are useful for ***isolating*** nucleic acid from a cell (claimed).

ADVANTAGE - Yields ***isolated*** ***purified*** nucleic acid freer form contaminants such as bacterial endotoxins than prior art methods. The methods reduce the viscosity of cell. . .

TECH. . . UPTX: 20010724

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: In M1 the solution comprising the biopolymer degrading enzyme and the ***hydrophobic*** surfactant are the same or different solutions and the cell is contacted with the enzyme first then the surfactant or both at the same time. The concentration of ***hydrophobic*** surfactant the cell suspension is at least 0.1%, 0.15%, or 0.2% (v/v) and has a critical micelle concentration of 0.1mM, 0.5mM, 1.0mM or 2.0mM. The ***hydrophobic*** surfactant has a hydrophile lipophile balance number of less than 20 or 15 and has a solubility of less than 2g, 1.5g or 1g/100ml in water. The solution comprising a ***hydrophobic*** surfactant further comprises a non- ***hydrophobic*** surfactant with a critical micelle concentration greater than 3.0mM, preferably 5.0mM or 7.0mM, and the concentration is at least 0.4% (v/v), preferably at least 0.5 or 0.6% (v/v). The non- ***hydrophobic*** surfactant has a hydrophile lipophile balance number of greater than 20 or 30 and a solubility greater than 2 g/100ml water. Preferably the ***hydrophobic*** surfactant is in a concentration of 0.2%(v/v) and the non- ***hydrophobic*** surfactant is 0.4% (v/v). The cell is a eukaryote or a prokaryote. M1 further comprises the step of ***isolating*** nucleic acid having an A260/A230 ratio of at least 2.0, and is preferably DNA, more preferably ***plasmid*** DNA. In M2 the cell is contacted with the ***hydrophobic*** surfactant for a period of 3 to 12 minutes before adding the neutralizing agent and the neutralizing agent is an acidic salt. M2 further comprises adding a non- ***hydrophobic*** surfactant as in M1.

Preferred Enzyme: The biopolymer degrading enzyme is a carbohydrate degrading, protein degrading or lipid-degrading enzyme preferably a carbohydrate degrading enzyme more preferably alpha-amylase, beta-amylase, amyloglucosidase, invertase, and glycopeptidase F.

Preferred Nucleic Acid: The ***isolated*** DNA preparation is preferably 90% or 95% ***supercoiled*** DNA.

TECHNOLOGY FOCUS - ORGANIC CHEMISTRY - The ***hydrophobic*** surfactant is polyoxoethylene sorbitan monolaureate, polyoxoethylene sorbitan monooleate, Triton X-100, Triton X-114, N-tetradecyl-N, N-dimethyl-3-ammonio-1-propanesulfonate, sodium dioctyl sulfosuccinate, surfynol(RTM) 420, surfynol(RTM) 440, surfynol(RTM) 465, surfynol(RTM) 485 and TR-70.

The non- ***hydrophobic*** surfactant is sodium dodecyl sulfate or CHAPS.

TT TT: ***ISOLATE*** NUCLEIC ACID MOLECULAR CELL COMPRISE CONTACT CELL SOLUTION CONTAIN DEGRADE ENZYME ***HYDROPHOBIC*** SURFACTANT YIELD CELL SUSPENSION.

L19 ANSWER 2 OF 9 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

ACCESSION NUMBER: 2001-049925 [06] WPIDS

DOC. NO. CPI: C2001-013739

TITLE: ***Purifying*** ***plasmid*** DNA, useful in gene therapy, by selective retention of impurities, particularly endotoxin, on ***hydrophobic*** interaction resin.

DERWENT CLASS: A96 B04 D16

INVENTOR(S): RAMASUBRAMANYAN, N

PATENT ASSIGNEE(S): (BIOS-N) BIO SCI CONTRACT PRODN CORP

COUNTRY COUNT: 91

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000073318	A1	20001207	(200106)*	EN	58

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW

W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

AU 2000051655 A 20001218 (200118)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000073318	A1	WO 2000-US14527	20000526
AU 2000051655	A	AU 2000-51655	20000526

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2000051655	A Based on	WO 200073318

PRIORITY APPLN. INFO: US 1999-136772 19990528

TI ***Purifying*** ***plasmid*** DNA, useful in gene therapy, by selective retention of impurities, particularly endotoxin, on ***hydrophobic*** interaction resin.

AB WO 200073318 UPAB: 20010126

NOVELTY - ***Purification*** of ***plasmid*** DNA (I) from a mixture containing at least 1 host cell impurity (II) by adding enough salt to the mixture to allow selective binding of at least 1 (II) to a ***hydrophobic*** interaction medium (III), treating the resulting solution with (III) so that (II) binds to form a complex, and recovering unbound. . . detergents, glycols, hexamine cobalt, spermidine or poly(vinyl pyrrolidone).

INDEPENDENT CLAIMS are also included for the following:

(a) a method for ***separating*** ***supercoiled*** ***plasmid*** DNA (Ia) from a mixture containing relaxed ***plasmid*** DNA (Ib) and optionally at least 1 (II);

(b) a method for ***separation*** of endotoxin (IIa) from (I) by treatment with (III) to form a complex with (IIa);

(c) a method for enriching the amount of (Ia) relative to (Ib); and

(d) a method for ***separating*** lipopolysaccharide (LPS) from a DNA-containing composition.

USE - The method is especially used to remove endotoxin (lipopolysaccharide), but also RNA, chromosomal DNA and protein, from ***plasmid*** DNA (or DNA generally, including cosmids and phagemids), particularly where the ***plasmids*** are intended for gene therapy and have been grown in bacteria.

ADVANTAGE - (III) has a much higher selectivity for

lipopolysaccharide and lipoproteins than for DNA over a wide pH range and
also allows ***separation*** of ***supercoiled*** and relaxed ***plasmids*** . Typically the impurity contents are reduced to 1-300 endotoxin units/mg DNA, to below 0.1wt.% for protein and to below 1wt.%.
. . processes and the preferred (III) have exceptionally high capacity for endotoxin (over 1 million units/ml), allowing over 95% recovery of ***plasmid*** DNA.
Dwg.0/7
TECH. . .
about 2 M, and the solution has a pH of 6.8-7.4, particularly 7.4. (III) is a chromatography support with pendant ***hydrophobic*** groups, preferably 3-10C alkyl. Most preferably it has a methacrylate-ethylene glycol copolymer or crosslinked agarose backbone, and is especially in. .
.
TT TT: ***PURIFICATION*** ***PLASMID*** DNA USEFUL GENE THERAPEUTIC
SELECT RETAIN IMPURE ENDOTOXIN ***HYDROPHOBIC*** INTERACT RESIN.

L19 ANSWER 3 OF 9 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000177966 EMBASE
TITLE: ***Purification*** of a cystic fibrosis ***plasmid*** vector for gene therapy using ***hydrophobic*** interaction chromatography.
AUTHOR: Diogo M.M.; Queiroz J.A.; Monteiro G.A.; Martins S.A.M.;
Ferreira G.N.M.; Prazeres D.M.F.
CORPORATE SOURCE: D.M.F. Prazeres, Ctro. Engenharia Biologica e Quimica,
Instituto Superior Tecnico, Av. Rovisco Pais, 1000 Lisboa, Portugal. prazeres@alfa.ist.utl.pt
SOURCE: Biotechnology and Bioengineering, (5 Jun 2000) 68/5 (576-583).
Refs: 18
ISSN: 0006-3592 CODEN: BIBIAU
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
TI ***Purification*** of a cystic fibrosis ***plasmid*** vector for gene therapy using ***hydrophobic*** interaction chromatography.
AB . . . and DNA vaccination in in vivo experiments and human clinical trials depend on the ability to produce large amounts of ***plasmid*** DNA according to defined specifications. A new method is described for the
purification of a cystic fibrosis ***plasmid*** vector (pCF1-CFTR) of clinical grade, which includes an ammonium sulfate precipitation
followed by ***hydrophobic*** interaction chromatography (HIC) using a
Sepharose gel derivatized with 1,4-butanedioldiglycidylether. The use of HIC took advantage of the more ***hydrophobic*** character of single-stranded nucleic acid impurities as compared with double-stranded ***plasmid*** DNA. RNA, denatured genomic and ***plasmid*** DNAs,
with large stretches of single strands, and lipopolysaccharides (LPS) that are more ***hydrophobic*** than super-coiled ***plasmid*** , were retained and ***separated*** from non-binding ***plasmid*** DNA in
a 14-cm HIC column. Anion-exchange HPLC analysis proved that >70% of the
loaded ***plasmid*** was recovered after HIC. RNA and denatured ***plasmid*** in the final ***plasmid*** preparation were undetectable by agarose electrophoresis. Other impurities, such as host genomic DNA and LPS, were reduced to residual values. . . detected in the final preparation by bicinchoninic acid (BCA) assay and sodium dodecylsulfate- polyacrylamide gel electrophoresis (SDS-PAGE) with silver
staining. ***Plasmid*** identity was confirmed by restriction analysis and biological activity by transformation experiments. The process presented constitutes an advance over existing. . .
CT Medical Descriptors:
*cystic fibrosis: TH, therapy
****plasmid vector***
*gene therapy
*chromatography
hydrophobicity
derivatization
anion exchange chromatography
high performance liquid chromatography
precipitation
polyacrylamide gel electrophoresis
DNA supercoiling
nonhuman
article
****plasmid DNA***
sepharose
1,4 butanediol
single stranded DNA
lipopolysaccharide
ammonium acetate

L19 ANSWER 4 OF 9 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:327378 HCAPLUS
TITLE: Novel chromatographic resins for the ***purification*** of ***supercoiled*** ***plasmid*** DNA.
AUTHOR(S): O'Donnell, J. Kevin; Fisher, Jon R.; Picciotti, Robert A.; Yamasaki, Oscar
CORPORATE SOURCE: Technical Service, TosoHaas, Montgomeryville, PA,
18936, USA
SOURCE: Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000 (2000), BIOT-153. American Chemical Society: Washington, D. C.
CODEN: 69CLAC
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
TI Novel chromatographic resins for the ***purification*** of ***supercoiled*** ***plasmid*** DNA.
AB Gene Therapy continues to grow as a new discipline in the pharmaceutical
industry. Of utmost importance is the ***purifn*** . of the vector whether it be viral or nonviral in nature. The marriage of chromatog. techniques with viral and DNA ***purifn*** . at scales large enough to support clin. trials has been rather arduous. Recently however, the application of ***Hydrophobic*** Interaction Chromatog. for the ***purifn*** . of ***plasmids*** was reported. Using a very ***hydrophobic*** chromatog. resin, Toyopearl Hexyl-650C, the sepn. of
supercoiled from open circular was accomplished with std. lab. conditions. This resin has a hexyl ligand covalently attached to a very hydrophilic polymethacrylate backbone. The pore size is nominally 1000.ANG. and the particle size is 50-150.mu.m. New chromatog. resins contg. even larger pores were synthesized to accommodate the size of gene
therapy vectors. These resins include both ion exchange and ***hydrophobic*** interaction modes. Investigating the effect of pore size and particle size will help to optimize the resoln. and dynamic binding capacity of ***supercoiled*** ***plasmids*** on chromatog.
resins. Studies are currently underway to quickly and efficiently characterize the sepn. of bacterial host proteins from the desired ***supercoiled*** ***plasmid*** using techniques that do not require
the use of org. solvents or added proteins.

L19 ANSWER 5 OF 9 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:688475 HCAPLUS
DOCUMENT NUMBER: 132:61133
TITLE: ***Separation*** and Analysis of ***Plasmid*** Denatured Forms Using ***Hydrophobic*** Interaction Chromatography
AUTHOR(S): Diogo, M. M.; Queiroz, J. A.; Monteiro, G. A.; Prazeres, D. M. F.
CORPORATE SOURCE: Centro de Engenharia Biologica e Quimica, Instituto
Superior Tecnico, Lisbon, 1000, Port.
SOURCE: Anal. Biochem. (1999), 275(1), 122-124
CODEN: ANBCA2; ISSN: 0003-2697
PUBLISHER: Academic Press

DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 5
REFERENCE(S): (1) Bimboim, H; Nucleic Acids Res 1979, V7, P1513
HCAPLUS
(2) Prazeres, D; J Chromatogr A 1998, V806, P31
HCAPLUS
(3) Queiroz, J; J Chromatogr A 1995, V707, P137
HCAPLUS
(4) Sayers, J; Anal Biochem 1996, V241, P186 HCAPLUS
(5) Sundberg, L; J Chromatogr 1974, V90, P87 HCAPLUS
TI ***Separation*** and Analysis of ***Plasmid*** Denatured Forms Using ***Hydrophobic*** Interaction Chromatography
AB This work explores the possibility of using a ***hydrophobic*** interaction chromatog. (HIC) support to sep. ***supercoiled*** ***plasmids*** from denatured forms, by taking advantage of their different surface hydrophobicity. The ***hydrophobic*** gel used in this work was prepd. by covalent immobilization of 1,4-butanediol diglycidyl ether on Sepharose CL-6B (Pharmacia). The ***hydrophobic*** interaction between this support and lipases was previously reported. Expts. were carried out in a 16 x 150-mm column packed with this gel and equilibrated with 10 mM Tris, pH 8, with 1.5 M (NH4)2SO4 at a flow rate of 60 mL/h. The absorbance was monitored at 254 nm. The ***plasmid*** used in the expts. was produced by fermn. of E. coli DH5.alpha. competent cells transformed with the 8.5-kb pCF1-CFTR ***plasmid*** (Genzyme Corp.). Growth was carried out overnight in LB medium (30,ug/mL kanamycin), in 100-mL shake-flasks at 37.degree. and 250 rpm. This work shows that HIC can be used for the sepn. of ***plasmid*** variants. The technique can play an important role in the preparative ***purifn*** . of super-coiled ***plasmids*** for gene therapy and DNA vaccination. In fact, the HIC support studied was capable of removing denatured ***plasmid*** variants that are usually produced with the widespread method of alk. lysis of ***plasmid*** ***isolation*** . This is very difficult to achieve using other chromatog. processes. Another important application could be in the monitoring and quality control of ***purified*** ***plasmids*** . Ongoing work indicates also an ability of the HIC support to sep. RNA and genomic DNA from ***plasmids*** . (c) 1999 Academic Press.
ST ***hydrophobic*** interaction chromatog ***plasmid*** ***purifn***
IT ***Plasmids***
(pCF1-CFTR; sepn. and anal. of ***plasmid*** denatured forms using ***hydrophobic*** interaction chromatog.)
IT Escherichia coli
Fermentation
Gene therapy
Hydrophobic interaction chromatography
Immobilization, biochemical
Liquid chromatographic stationary phases
Quality control
(sepn. and anal. of ***plasmid*** denatured forms using ***hydrophobic*** interaction chromatog.)
IT Immunization
(vaccination; sepn. and anal. of ***plasmid*** denatured forms using ***hydrophobic*** interaction chromatog.)
IT 2425-79-8, 1,4-Butanediol diglycidyl ether 62610-50-8, Sepharose CL 6B
RL: ARU (Analytical role, unclassified); RCT (Reactant); ANST (Analytical study)
(sepn. and anal. of ***plasmid*** denatured forms using ***hydrophobic*** interaction chromatog.)

L19 ANSWER 6 OF 9 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1999156202 EMBASE
TITLE: Capillary gel electrophoresis of nucleic acids in pluronic F127 copolymer liquid crystals.
AUTHOR: Rill R.L.; Liu Y.; Ramey B.A.; Van Winkle D.H.; Locke B.R.
CORPORATE SOURCE: R.L. Rill, Department of Chemistry, Institute of Molecular Biophysics, Florida State University, Tallahassee, FL, United States
SOURCE: Chromatographia, (1999) 49/SUPPL. 1 (S65-S71). Refs: 20
ISSN: 0009-5893 CODEN: CHRGB7
COUNTRY: Germany
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB . . . approximate formula (EO)106 (PO)70 (EO)106. Polymer chains aggregate into spherical micelles in aqueous solutions, with poly(propylene oxide) chains creating a ***hydrophobic*** core surrounded by brushes of hydrated poly(ethylene oxide) tails. Crowding at high concentrations promotes ordering of micelles. Solutions in the. . . viscosity liquids that are easily loaded into capillaries. This article reviews applications of Pluronic F127 media for capillary gel electrophoresis ***separations*** of nucleic acids of several types including oligonucleotides, double stranded DNA fragments, and ***supercoiled*** ***plasmid*** DNAs.

L19 ANSWER 7 OF 9 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1998278790 EMBASE
TITLE: Pluronic copolymer liquid crystals: Unique, replaceable media for capillary gel electrophoresis.
AUTHOR: Rill R.L.; Liu Y.; Van Winkle D.H.; Locke B.R.
CORPORATE SOURCE: R.L. Rill, Department of Chemistry, Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306-4390, United States
SOURCE: Journal of Chromatography A, (1998) 817/1-2 (287-295). Refs: 37
ISSN: 0021-9673 CODEN: JCRAEY
PUBLISHER IDENT.: S 0021-9673(98)00522-6
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB . . . oxide) [(EO)(x)] and poly(propylene oxide) [(PO)(y)] with the general formula (EO)(x)(PO)(y)(EO)(x). Large micelles form in aqueous solutions in which central, ***hydrophobic*** cores of (PO)(y) segments are surrounded by “brushes” of hydrated (EO)(x) tails. Solutions of Pluronic F127 (BASF Performance Chemicals) in. . . of spherical micelles with diameters of 17-18 nm which pack with local cubic symmetry. CGE in Pluronic F127 liquid crystals ***separates*** species within several chemical classes as varied as nucleoside monophosphates and organic dyes, oligonucleotides of 4-60 nucleotides, DNA fragments of 50-3000 base pairs (bp), and ***supercoiled*** ***plasmid*** DNAs of 2000-10 000 bp. Mechanisms of molecular sieving in polymer liquid crystals must differ in fundamental ways from ***separations*** in random polymer gels because molecules move around uncrosslinked obstacles that are larger than the smallest dimensions of typical analytes.. . . (EO)(x) strands of micelle brushes, or through brushtips and interstitial spaces between micelles. Small molecules such as nucleotides appear to ***separate*** by a different mechanism involving partitioning between hydrophilic and ***hydrophobic*** environments. This process is termed “ ***hydrophobic*** interaction electrophoresis”. The unique structures of Pluronic copolymers and their liquid crystalline phases provide new challenges and opportunities in ***separations*** science. Copyright (C) 1998 Elsevier Science B.V.

L19 ANSWER 8 OF 9 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 92337651 EMBASE
DOCUMENT NUMBER: 1992337651
TITLE: Preparation of DNA topoisomers by RP-18 high-performance liquid chromatography.

AUTHOR: Kapp U.; Langowski J.
CORPORATE SOURCE: EMBL, Grenoble Outstation, P.O. Box 156X,F-38042 Grenoble Cedex, France
SOURCE: Analytical Biochemistry, (1992) 206/2 (293-299). ISSN: 0003-2697 CODEN: ANBCA2
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A method for the ***separation*** of superhelical DNA on the basis of superhelical density by reverse-phase HPLC on RP-18 columns is described. The technique can. . . be used to prepare superhelical DNA in milligram amounts and narrow topoisomer distributions in 0.1 mg amounts. We show example ***separations*** of the ***plasmids*** pUC18 (2687 bp) and .pi.AN13 (895 bp). While the best ***separation*** for pUC18 yields topoisomer distributions of two or three major components, the small ***plasmid*** can be ***separated*** into single topoisomer fractions. The basis of the ***separation*** is probably an interaction of partially opened bases with the ***hydrophobic*** column matrix. This hypothesis is supported by the elution behavior of DNA fragments on this column: DNA fragments with sticky. . .
CT Medical Descriptors:
*dna determination
*reversed phase high performance liquid chromatography
animal cell
article
cell separation
column chromatography
dna structure
dna supercoiling
hydrophobicity
intercalation complex
ligand binding
nonhuman
plasmid
priority journal
*dna topoisomerase: EC, endogenous compound

L19 ANSWER 9 OF 9 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1986:279543 BIOSIS
DOCUMENT NUMBER: BA82:23406
TITLE: ANALYSIS AND ***PURIFICATION*** OF ***PLASMID*** DNA BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY.
AUTHOR(S): COLOTE S; FERRAZ C; LIAUTARD J P
CORPORATE SOURCE: UNITE U. 249 INSERM, INST. DE BIOL., BLVD. HENRI IV, 34000 MONTPELLIER.
SOURCE: ANAL BIOCHEM, (1986) 154 (1), 15-20. CODEN: ANBCA2. ISSN: 0003-2697.
FILE SEGMENT: BA; OLD
LANGUAGE: English
TI ANALYSIS AND ***PURIFICATION*** OF ***PLASMID*** DNA BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY.
AB Large nucleic acids can be ***separated*** by reversed-phase high-performance liquid chromatography. Under our experimental conditions, the retention time depends not on the chain length but rather on the base composition and the secondary structure of the molecule. Because of the torsional strain caused by the ***supercoiling*** of the ***plasmid*** , more of its bases are accessible for interaction with the ***hydrophobic*** stationary phase. This increases the retention time of the ***supercoiled*** DNA compared to the relaxed or linear DNA. We have exploited these properties to analyze the quality of ***plasmid*** preparations. The method is more sensitive to contaminants than common electrophoretic techniques. Furthermore, we describe a convenient and rapid procedure for ***purifying*** ***plasmid*** DNA. The highly pure ***plasmid*** is biologically more active for most of the enzymatic reactions commonly used in genetic engineering.

(FILE 'HOME' ENTERED AT 09:45:16 ON 16 SEP 2001)

FILE 'BIOSIS, EMBASE, MEDLINE, WPIDS, HCAPLUS' ENTERED AT 09:45:30 ON 16 SEP 2001
L1 18040 S SUPERCOIL?
L2 236069 S HYDROPHOBIC
L3 5628458 S PURIF? OR SEPARAT? OR ISOLAT?
L4 35 S L1 AND L2 AND L3
L5 19 DUP REM L4 (16 DUPLICATES REMOVED)
L6 1781609 S PURIF?
L7 1730365 S SEPARAT?
L8 3039314 S ISOLAT?
L9 23 S L1 AND L2 AND L6
L10 20 S L1 AND L2 AND L7
L11 15 S L1 AND L2 AND L8
L12 35 S L9 OR L10 OR L11
L13 19 DUP REM L12 (16 DUPLICATES REMOVED)
L14 157356 S ENDOTOXIN OR LPS
L15 2210 S REVERSE(W)PHASE(W)CHROMATOGRAPHY
L16 0 S L1 AND L15
L17 315171 S PLASMID?
L18 9 S L5 AND L17
L19 9 DUP REM L18 (0 DUPLICATES REMOVED)
L20 2362002 S REMOV?
L21 13289 S L14 AND L6
L22 4191 S L14 AND L7
L23 19717 S L14 AND L8
L24 5749 S L14 AND L20
L25 5 S L15 AND L14
L26 3 DUP REM L25 (2 DUPLICATES REMOVED)
L27 34907 S L21 OR L22 OR L23 OR L24
L28 478 S L27 AND L2
L29 27971 S L2 AND CHROMATOG?
L30 152 S L27 AND L29
L31 24062 S L2(L)CHROMATOG?
L32 153 S L14 AND L31

=> s l31 and l24
L33 25 L31 AND L24

=> dup rem
ENTER L# LIST OR (END):l33
PROCESSING COMPLETED FOR L33
L34 14 DUP REM L33 (11 DUPLICATES REMOVED)

=> s l26 or l34
L35 17 L26 OR L34

=> d l35 ibib kwic 1-17

L35 ANSWER 1 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2001:356637 BIOSIS
DOCUMENT NUMBER: PREV200100356637
TITLE: ***Removal*** of tightly bound ***endotoxin*** from biological products.
AUTHOR(S): Wilson, Mark J. (1); Haggart, Claire L.; Gallagher, Sean P.; Walsh, Deirdre
CORPORATE SOURCE: (1) Xenova, Downstream Process Development, Milton Road, 310 Cambridge Science Park, Cambridge, CB4 0WG: mwilson@cantab.co.uk UK
SOURCE: Journal of Biotechnology, (1 June, 2001) Vol. 88, No. 1, pp. 67-75. print. ISSN: 0168-1656.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
TI ***Removal*** of tightly bound ***endotoxin*** from biological products.
AB The method for ***endotoxin*** ***removal*** described in this paper is useful for separation of tightly bound ***endotoxin*** from biological products, particularly those produced in Escherichia coli in

the form of inclusion bodies for which a denaturation step is required to solubilise the product. We employed guanidine hydrochloride and ammonium
sulphate in combination with ***hydrophobic*** interaction ***chromatography*** (HIC). These conditions enable binding of the ***endotoxin*** to the matrix, giving unbound product in the column flow-through. This makes the method generally applicable to biological products. An ***endotoxin*** reduction of about 3.7 logs was achieved;
from as much as 1 100 000 EU mg-1 in the solubilised material. . . protein comprising L2, E7 and E6 from Human Papilloma Virus type 16, because both conventional and commercially available methods of ***endotoxin*** ***removal*** were ineffective in ***removing***
the tightly bound ***endotoxin*** from this product.
IT Major Concepts
Biochemistry and Molecular Biophysics; Bioprocess Engineering; Pharmacology; Toxicology
IT Chemicals & Biochemicals
TA-CIN: purification, vaccine; ammonium sulfate; ***endotoxin*** : tightly-bound, toxin; guanidine hydrochloride; inclusion bodies
IT Methods & Equipment
hydrophobic interaction ***chromatography*** [HIC]: liquid ***chromatography*** , separation method
IT Miscellaneous Descriptors
hydrophobic interaction

L35 ANSWER 2 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1999:502729 BIOSIS
DOCUMENT NUMBER: PREV199900502729
TITLE: Efficient method for preparation of highly purified lipopolysaccharides by ***hydrophobic*** interaction ***chromatography*** .
AUTHOR(S): Muck, Andreas; Ramm, Michael; Hamburger, Matthias (1)
CORPORATE SOURCE: (1) Pharmazeutische Biologie, Friedrich-Schiller-Universitaet, Institut fuer Pharmazie, Semmelweisstrasse 10, Jena, D-07743 Germany
SOURCE: Journal of Chromatography B, (Sept. 10, 1999) Vol. 732, No.
1, pp. 39-46.
ISSN: 0378-4347.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
TI Efficient method for preparation of highly purified lipopolysaccharides by ***hydrophobic*** interaction ***chromatography*** .
AB A method for the efficient preparation of highly purified lipopolysaccharides (LPSs) by ***hydrophobic*** interaction ***chromatography*** (HIC) has been developed. The procedure can be used
for the purification of cell wall bound LPSs after hot phenol-water. . . polysaccharide, protein and RNA and subsequently employed for the preparative purification of two LPSs of different origin, namely the extracellular ***LPS*** secreted by Escherichia coli E49 into the culture medium, and the cell wall bound ***LPS*** from Pseudomonas aeruginosa VA11465/1. Compared to currently used methods for ***LPS***
purification such as enzymatic digestion and ultracentrifugation, the ***chromatographic*** separation reported here combines superior purity
with minimal loss of ***LPS*** , high reproducibility and simple handling. The ***removal*** of contaminants such as protein, RNA and
polysaccharides and the recovery of LPSs were monitored by appropriate assays.
IT Methods & Equipment
fermentation: synthesis/modification techniques, synthetic method; hot phenol-water extraction: Isolation/Purification Techniques: cb, purification method; ***hydrophobic*** interaction ***chromatography*** : liquid ***chromatography*** , purification method; 5-1 Biostat B laboratory fermentor: B. Braun Biotech, laboratory equipment

L35 ANSWER 3 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1994:527661 BIOSIS
DOCUMENT NUMBER: PREV199497540661
TITLE: Stimulators of tumour necrosis factor production released by damaged erythrocytes.
AUTHOR(S): Bate, C. A. W. (1); Kwiatkowski, D. P.
CORPORATE SOURCE: (1) Dep. Paediatr., Inst. Molecular Med., John Radcliffe
Hosp., Headington, Oxford OX3 9DU UK
SOURCE: Immunology, (1994) Vol. 83, No. 2, pp. 256-261.
ISSN: 0019-2805.
DOCUMENT TYPE: Article
LANGUAGE: English
AB. . . cells (PBMNC) to release tumour necrosis factor-alpha (TNF). This response is not inhibited by polymyxin B, indicating that contaminating lipopolysaccharide (***LPS***) is not responsible. When erythrocyte lysates are fractionated by ***reverse*** - ***phase*** ***chromatography*** using a gradient of n-propanol on Sep-Pak C18 cartridges, the TNF-inducing activity elutes as a single peak. The erythrocyte-derived TNF-inducing. . . also inhibits the TNF response to erythrocyte-derived factors and to parasite lysates whereas it does not affect the response to ***LPS*** . We conclude that lysed erythrocytes release specific cytokine-inducing factors that may contribute to the fever response to non-infectious tissue injury.

L35 ANSWER 4 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1981:211521 BIOSIS
DOCUMENT NUMBER: BA71:81513
TITLE: PROPERTIES OF BINDING OF ESCHERICHIA-COLI ENDO TOXIN TO
VARIOUS MATRICES.
AUTHOR(S): MAITRA S K; YOSHIKAWA T T; GUZE L B; SCHOTZ M C
CORPORATE SOURCE: UCLA SCH. MED., LOS ANGELES, CALIF. 90024, USA.
SOURCE: J CLIN MICROBIOL, (1981) 13 (1), 49-53.
CODEN: JCMIDW. ISSN: 0095-1137.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Binding of E. coli O127:B8 ***endotoxin*** to a variety of resins and
column materials was investigated by measuring the .beta.-hydroxy myristic
acid content (a major component of the lipid A moiety) of ***endotoxin*** after hydrolysis by selected ion-monitoring gas ***chromatography*** -mass spectrometry. More than 80% of the ***endotoxin*** was bound to hydroxylapatite, polystyrene, Dowex 1-X2
and charcoal. The binding of ***endotoxin*** to these materials was markedly reduced by the addition of normal or delipidated [dog] serum. Phenyl- and octyl-Sepharose bound 56 and 50% of the ***endotoxin*** from saline solutions, respectively. Their percent binding was increased significantly in 1 M ammonium sulfate solutions, indicating ***hydrophobic*** interactions between ***endotoxin*** and phenyl-
and octyl-Sepharose. Only 5% of the ***endotoxin*** was bound to plastic polymer PSI-HAP-100 beads, and no binding was observed with concanavalin A- and heparin-Sepharose. Study of the in vitro binding of ***endotoxin*** to the above materials in the presence of serum suggests
that the use of these materials in ***removing*** circulating ***endotoxin*** in vivo is limited.

L35 ANSWER 5 OF 17 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 94284028 EMBASE
DOCUMENT NUMBER: 1994284028
TITLE: Purification of recombinant human granulocyte-macrophage colony-stimulating factor from the inclusion bodies produced by transformed Escherichia coli cells.
AUTHOR: Belew M.; Zhou Y.; Wang S.; Nystrom L.-E.; Janson J.-C.
CORPORATE SOURCE: Pharmacia Bioprocess Technology,S-751 82 Uppsala, Sweden
SOURCE: Journal of Chromatography A, (1994) 679/1 (67-83).
ISSN: 0021-9673 CODEN: JCRAEY
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB . . . colony-stimulating factor (rhGM-CSF), produced as inclusion bodies in genetically transformed Escherichia coli cells was purified to homogeneity by a three-step ***chromatographic*** procedure involving
hydrophobic interaction, ion exchange and gel filtration. Each purification step is reproducible and well suited for process-scale operations. The purification process also leads to a significant decrease in DNA and ***endotoxin*** levels in the final product. Of the three gel media used, Phenyl Sepharose 6 FF (high sub) was most effective. . . in reducing the DNA content (by a factor of ca. 2000) while Superdex 75 prep grade was more effective for ***removing*** endotoxins (reduction
factor ca. 15). The recovery of purified rhGM-CSF was 35% by enzyme-linked
immunosorbent assay and 70% by a. . .

L35 ANSWER 6 OF 17 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2001-049925 [06] WPIDS
DOC. NO. CPI: C2001-013739
TITLE: Purifying plasmid DNA, useful in gene therapy, by selective retention of impurities, particularly ***endotoxin*** , on hydrophobic interaction resin.
DERWENT CLASS: A96 B04 D16
INVENTOR(S): RAMASUBRAMANYAN, N
PATENT ASSIGNEE(S): (BIOS-N) BIO SCI CONTRACT PRODN CORP
COUNTRY COUNT: 91
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000073318	A1	20001207	(200106)*	EN	58

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ
NL OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE ES
FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS
LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK
SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW
AU 2000051655 A 20001218 (200118)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000073318	A1	WO 2000-US14527	20000526
AU 2000051655	A	AU 2000-51655	20000526

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2000051655	A Based on	WO 200073318

PRIORITY APPLN. INFO: US 1999-136772 19990528
TI Purifying plasmid DNA, useful in gene therapy, by selective retention of impurities, particularly ***endotoxin*** , on hydrophobic interaction resin.
AB . . .
from a mixture containing relaxed plasmid DNA (Ib) and optionally at least
I (II);
(b) a method for separation of ***endotoxin*** (IIa) from (I) by treatment with (III) to form a complex with (IIa);
(c) a method for enriching the amount of (Ia) relative to (Ib); and
(d) a method for separating lipopolysaccharide (***LPS***) from a DNA-containing composition.
USE - The method is especially used to ***remove*** ***endotoxin*** (lipopolysaccharide), but also RNA, chromosomal DNA and
protein, from plasmid DNA (or DNA generally, including cosmids and phagemids), particularly where. . . wide pH range and also allows separation of supercoiled and relaxed plasmids. Typically the impurity contents are reduced to 1-300 ***endotoxin*** units/mg DNA, to below
0.1wt.% for protein and to below 1wt.% for RNA and chromosomal DNA. The
method is suitable for large or laboratory-scale processes and the preferred (III) have exceptionally high capacity for ***endotoxin*** (over 1 million units/ml), allowing over 95% recovery of plasmid DNA. Dwg.0/7
TECH. . .
chloride at 2-4, particularly about 2 M, and the solution has a pH of 6.8-7.4, particularly 7.4. (III) is a ***chromatography*** support with pendant ***hydrophobic*** groups, preferably 3-10C alkyl. Most preferably it has a methacrylate-ethylene glycol copolymer or crosslinked agarose backbone, and is especially in. . . to the solution, and then (III), under conditions where both (Ia) and (Ib) bind. Conditions are than changed, first to ***remove*** (Ib) and then to release (Ia). The various conditions are defined by AS concentrations, especially 2.5-4 M for equilibration, 2.35-2.45 M to ***remove*** (Ib) and then 1-2.3 M to recover (Ia). The AS concentration may be altered continuously by gradient elution. In method (b), ***endotoxin*** binds at AS concentration 1.5-4, preferably about 2, M. In method (c), the mixture of (Ia) and (Ib) is contacted with (III) under conditions where (Ia) binds preferentially, then (III) is treated under ionic conditions that allow preferential ***removal*** of (Ib), followed by elution of (Ia). In method (d), the DNA- ***LPS*** mixture is contacted with (III) containing an alkyl group under conditions where ***LPS*** binds preferentially, then treated under ionic conditions that allow selective ***removal*** of the DNA.

TECHNOLOGY FOCUS - POLYMERS - Preferred Method: (III) particularly
comprises a backbone of methacrylate-ethylene glycol copolymer, with. .
.
TT TT: PURIFICATION PLASMID DNA USEFUL GENE THERAPEUTIC SELECT RETAIN IMPURE
ENDOTOXIN HYDROPHOBIC INTERACT RESIN.

L35 ANSWER 7 OF 17 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1995-302501 [39] WPIDS
DOC. NO. CPI: C1995-135398
TITLE: Purifying human lactoferrin from milk - by contacting the milk with a strong cation exchange resin then eluting with a salt soln..
DERWENT CLASS: B04 D13 D16
INVENTOR(S): NUIJENS, J H; VAN VEEN, H H; VAN DEEN, H H; NULJENS, J H;
VAN VEEN, H; NUYENS, J H
PATENT ASSIGNEE(S): (GENE-N) GENE PHARMING EURO BV; (PHAR-N) PHARMING BV;
(PHAR-N) PHARMING INTELLECTUAL PROPERTY BV
COUNTRY COUNT: 62
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9522258	A2	19950824	(199539)*	EN	67

RW: AT BE CH DE DK ES FR GB GR IE IT KE LU MC MW NL OA PT SD SE SZ UG
W: AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU JP KE KG
KP KR KZ LK LR LT LU LV MD MG MN MW MX NL NO NZ PL PT RO RU SD SE
SI SK TJ TT UA UG US UZ VN
AU 9518098 A 19950904 (199549)
WO 9522258 A3 19951116 (199621)
FI 9603197 A 19960815 (199644)
NO 9603163 A 19960916 (199646)
EP 744901 A1 19961204 (199702) EN
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE
JP 09509165 W 19970916 (199747) 77
KR 97701006 A 19970317 (199813)
US 5849885 A 19981215 (199906)
US 5861491 A 19990119 (199911)
US 5919913 A 19990706 (199933)
MX 9603369 A1 19971201 (199936)
AU 9923687 A 19990826 (199946)
NZ 330484 A 19991129 (200031)
NZ 336981 A 20010629 (200140)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9522258	A2	WO 1995-EP583	19950216
AU 9518098	A	AU 1995-18098	19950216
WO 9522258	A3	WO 1995-EP583	19950216
FI 9603197	A	WO 1995-EP583	19950216
		FI 1996-3197	19960815
NO 9603163	A	WO 1995-EP583	19950216
		NO 1996-3163	19960729
EP 744901	A1	EP 1995-909735	19950216
		WO 1995-EP583	19950216
JP 09509165	W	JP 1995-521594	19950216
		WO 1995-EP583	19950216
KR 97701006	A	WO 1995-EP583	19950216
		KR 1996-704482	19960816
US 5849885	A Cont of	US 1994-198321	19940216
	Cont of	US 1995-406271	19950309
		US 1995-464182	19950605
US 5861491	A	WO 1995-EP583	19950216
		US 1996-693274	19961016
US 5919913	A Cont of	US 1994-198321	19940216
		US 1995-406271	19950309
MX 9603369	A1	MX 1996-3369	19960814
AU 9923687	A Div ex	AU 1995-18098	19950216
		AU 1999-23687	19990409
NZ 330484	A Div ex	NZ 1995-281219	19950216
		NZ 1995-330484	19950216
NZ 336981	A Div ex	NZ 1995-330484	19950216
		NZ 1995-336981	19950216

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9518098	A Based on	WO 9522258
EP 744901	A1 Based on	WO 9522258
JP 09509165	W Based on	WO 9522258
KR 97701006	A Based on	WO 9522258
US 5861491	A Based on	WO 9522258
NZ 330484	A Div ex	NZ 281219
NZ 336981	A Div ex	NZ 330484

PRIORITY APPLN. INFO: US 1994-198321 19940216; US 1995-406271 19950309; US 1995-464182 19950605; US 1996-693274 19961016

AB . . .

fraction contg. hLF with a strong cation exchange (SCE) resin under elevated ionic strength conditions to form hLF-resin complexes; (b) ***removing*** the milk or milk fraction which remains unbound and recovering the hLF-resin complexes; and (c) eluting the hLF from the. . . hLF; (D) recombinant hLF produced in the milk of a transgenic nonhuman animal and purified from the milk by SCE ***chromatography*** and/or a concanavalin A resin; (E) a method for ***removing*** lipopolysaccharide (***LPS***) from a soln. comprising contacting a soln. contg. ***LPS*** with a resin comprising immobilised hLF and recovering the portion of the soln. that is bound to the resin; (F) the use of ***hydrophobic*** interaction ***chromatography*** (HIC) in the sepn. of hLF from bLF; (G) an isolated mouse domferrin protein having the N-terminal sequence: Lys-Ala-Val-Arg-Val-Gln-Trp-XXX-Ala-Val-Ser-Asn-Glu-Glu; (X. . . method for the purificn. of a mouse domferrin protein which comprises subjecting a source of the domferrin to cation exchange ***chromatography*** ; (I) the use of anion exchange ***chromatography*** in purifying domferrin.

USE - The methods are used partic. for purifying hLF from bovine milk produced by a. . .

L35 ANSWER 8 OF 17 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1992-398124 [48] WPIDS
CROSS REFERENCE: 1988-338202 [47]
DOC. NO. CPI: C1992-176614

TITLE: Process for recovering pure recombinant interleukin-2 - comprises denaturation, oxidn. and chromatography in discrete steps, giving non-glycosylated denatured interleukin-2.
DERWENT CLASS: B04 D16
INVENTOR(S): DAVIS, J T; DORIN, G J; LIM, A; SMITH, F; WEISSBURG, R; WOLFE, S N
PATENT ASSIGNEE(S): (CETU) CETUS CORP
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 5162507	A	19921110 (199248)*			14

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
US 5162507	A CIP of	US 1987-48408	19870511
	CIP of	US 1988-167144	19880325
		US 1989-406365	19890912

PRIORITY APPLN. INFO: US 1987-48408 19870511; US 1988-167144 19880325; US 1989-406365 19890912

AB . . .

material in a sufficient amt. of a guanidine salt and reducing agent; (c) reducing the conc. of guanidine salt to ***remove*** IL-2 precipitates and sol. proteins; (d) re-dissolving the IL-2 ppte. in a sufficient amt. of a guanidine salt; (e) oxidising. . . (f) reducing the guanidine conc. to ppte insol. proteins from the transformant while IL-2 remains sol.; and (g) sepg. and ***removing*** the insol. proteins from the sol. IL-2.

Also claimed are methods for recovering purified rendured recobinant IL-2 from a transformant;. . . of IL-2; (g) purifying the oxidised IL-2 by either reverse phase HPLC followed by dissolution using a chaotropic agent, or ***hydrophobic*** interaction ***chromatography*** and ion-exchange ***chromatography*** ; or (3) ion-exchange ***chromatography*** ; (h) reducing the conc. of the chaotropic agent, allowing renaturation and precipitation of the IL-2; (i) sepg. the ppte from. . . least 5 mg IL-2/ml, specific activity of at leas 1 x 10 power (7) u/mg (HT-2 cell proliferation assay) and ***endotoxin*** content of below 0.1 ng/mg IL-2.

USE/ADVANTAGE - This method allows for the production of non-glycosylated, renatured IL-2. The oxidation. . .

L35 ANSWER 9 OF 17 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1990-265448 [35] WPIDS
DOC. NO. CPI: C1990-114856
TITLE: Purificn. of human B cell differentiation factor - obtd. from E. coli, by treating soln. contg. factor by 2 stage ***reverse*** ***phase*** ***chromatography*** .
DERWENT CLASS: B04 D16
PATENT ASSIGNEE(S): (AJIN) AJINOMOTO KK
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
JP 02186996	A	19900723 (199035)*			

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
JP 02186996	A	JP 1989-5769	19890112

PRIORITY APPLN. INFO: JP 1989-5769 19890112

TI . . . Purificn. of human B cell differentiation factor - obtd. from E. coli, by treating soln. contg. factor by 2 stage ***reverse*** ***phase*** ***chromatography*** .

AB . . .

of human B-cell differentiation factor (BCDF) produced by E. coli, the human BCDF contg. soln. is treated by 2 stage ***reverse***

phase ***chromatography*** to give a protein purity of human
BCDF of at least 99%, and content of ***endotoxin*** below 0.6 ***endotoxin*** unit/mg protein.
USE/ADVANTAGE - Human BCDF is purified simply and in good efficiency.
In an example, fused protein. . .
TT TT: PURIFICATION HUMAN CELL DIFFERENTIAL FACTOR OBTAIN COLI TREAT SOLUTION
CONTAIN FACTOR STAGE ***REVERSE*** ***PHASE*** ***CHROMATOGRAPHY*** .

L35 ANSWER 10 OF 17 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1988-338202 [47] WPIDS
CROSS REFERENCE: 1992-398124 [48]
DOC. NO. CPI: C1988-149522
TITLE: Pure, renatured, recombinant interleukin 2 recovery - by treating refractile bodies with reducing agent under denaturing conditions, controlled oxidn., denaturing and chromatographic purifcn..
DERWENT CLASS: B04 D16
INVENTOR(S): DAVIS, J T; DORIN, G; LIM, A; SMITH, F; WOLFE, S N
PATENT ASSIGNEE(S): (CETU) CETUS CORP; (CHIR) CHIRON CORP; (CETU) CETUS ONCOLOGY CORP
COUNTRY COUNT: 19
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 8808849	A	19881117	(198847)*	EN	43
RW: AT BE CH DE FR GB IT LI LU NL SE					
W: AU DK FI HU JP NO					
AU 8816279	A	19881206	(198913)		
NO 8805678	A	19890227	(198914)		
DK 8900101	A	19890111	(198922)		
EP 368857	A	19900523	(199021)		
R: AT BE CH DE DK FI FR GB IT LI LU NL SE					
JP 02503384	W	19901018	(199048)		
NO 176797	B	19950220	(199512)		
CA 1337671	C	19951128	(199608)		
IL 86326	A	19970218	(199720)		
EP 368857	B1	19970806	(199736)	EN	129
R: AT BE CH DE FR GB IT LI LU NL SE					
DE 3855986	G	19970911	(199742)		
JP 2955294	B2	19991004	(199946)		15

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 8808849	A	WO 1988-US1043	19880331
EP 368857	A	EP 1988-903676	19880331
JP 02503384	W	JP 1988-503562	19880331
NO 176797	B	WO 1988-US1043	19880331
		NO 1988-5678	19881221
CA 1337671	C	CA 1988-563757	19880411
IL 86326	A	IL 1988-86326	19880510
EP 368857	B1	EP 1988-903676	19880331
		WO 1988-US1043	19880331
DE 3855986	G	DE 1988-3855986	19880331
		EP 1988-903676	19880331
		WO 1988-US1043	19880331
JP 2955294	B2	JP 1988-503562	19880331
		WO 1988-US1043	19880331

FILING DETAILS:

PATENT NO	KIND	PATENT NO
NO 176797	B Previous Publ.	NO 8805678
EP 368857	B1 Based on	WO 8808849
DE 3855986	G Based on	EP 368857
	Based on	WO 8808849
JP 2955294	B2 Previous Publ.	JP 02503384
	Based on	WO 8808849

PRIORITY APPLN. INFO: US 1988-167144 19880325; US 1987-48408 19870511
AB . . .
natural disulphide bridges; (7) reducing concn. of (B) so that oxidised IL-2 is renatured and a ppte. is formed (and ***removed***); (8) purifying oxidised IL-2 in the supernatant by (a)reverse-phase h.p.l.c., then dissolving in a soln. of (B) and ***removing*** (B); (b) ***hydrophobic*** interaction ***chromatography*** plus ion-exchange ***chromatography*** (IEc) or (c) IEC alone; and (9) recovering pure, oxidised, soluble, heterologous IL-2 of at least 95% purity (SDS-PAGE) with. . . phosphate buffered saline at least 5 mg./ml; specific activity at least 10 million units/mg. (by HT-2 cell proliferation assay) and ***endotoxin*** content below 0.1 ng./mg. The purified recombinant IL-2 is itself claimed.
USE/ADVANTAGE - This method provides high yields of IL-2. . .
ABEQ. . .
and refold into the configuration of native IL-2 and at which insoluble extraneous host proteins precipitate; and (g) separating and ***removing*** said insoluble proteins from the soluble renatured, oxidised IL-2.
Dwg.0/2

L35 ANSWER 11 OF 17 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1987-334948 [47] WPIDS
DOC. NO. CPI: C1987-142952
TITLE: Purifying immunogenic plasmodium polypeptide - from recombinant cell cultures by selective pptn., then ion exchange and ***reverse*** ***phase*** ***chromatography*** .
DERWENT CLASS: B04 D16
INVENTOR(S): DEPHILLIPS, P A; FOLENAWASS, G M; SITRIN, R D; ZABRISKIE, D W
PATENT ASSIGNEE(S): (DPHI-I) DE PHILLIPS P A; (SMIK) SMITHKLINE BECKMAN CORP
COUNTRY COUNT: 21
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 8706939	A	19871119	(198747)*	EN	40
W: AU DK FI JP NO US					
EP 252588	A	19880113	(198802)	EN	
R: AT BE CH DE ES FR GB GR IT LI LU NL SE					
AU 8774400	A	19871201	(198809)		
NO 8800080	A	19880321	(198817)		
PT 84850	A	19880527	(198826)		
DK 8800102	A	19880111	(198830)		
ZA 8703338	A	19880621	(198834)		
FI 8805237	A	19881111	(198931)		
JP 01502556	W	19890907	(198942)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 8706939	A	WO 1987-701115	19870511
EP 252588	A	EP 1987-304133	19870508
ZA 8703338	A	ZA 1987-3338	19870511
JP 01502556	W	JP 1987-503190	19870511

PRIORITY APPLN. INFO: US 1986-861810 19860512
TI Purifying immunogenic plasmodium polypeptide - from recombinant cell cultures by selective pptn., then ion exchange and ***reverse*** ***phase*** ***chromatography*** .
AB . . .
The purified material contains no measurable amts of unwanted polypeptides and proteins, not over 2ng/mg DNA and less than 10 ***endotoxin*** units/mg.
0/0
TT TT: PURIFICATION IMMUNOGENIC PLASMODIUM POLYPEPTIDE RECOMBINATION CELL

CULTURE SELECT PRECIPITATION ION EXCHANGE ***REVERSE*** ***PHASE*** ***CHROMATOGRAPHY*** . AW: VACCINE.

L35 ANSWER 12 OF 17 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:327374 HCAPLUS
TITLE: Purification of SY161 for therapeutic application.
AUTHOR(S): Wan, Mindy; Downing, Heather; Rabideau, Susan M.; Conn, Greg; Schrimsher, Jeff; Koch, George; Moreadith, Randall M.
CORPORATE SOURCE: Purification Process Development, Covance Biotechnology Services Inc, Cary, NC, 27513, USA
SOURCE: Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000 (2000), BIOT-149. American Chemical Society: Washington, D. C. CODEN: 69CLAC
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB SY161 is a recombinant analog of Staphylokinase (S. aureus) that has fibrin-selective thrombolytic activity in vivo. However, its short half-life necessitates continuous infusion for efficacy. A monoPEGylated form of SY161 has a longer half-life (and presumably lower immunogenicity) which allows for single bolus administration, and is currently in Phase II clin. trials for the treatment of acute myocardial infarction. This presentation will describe the recovery, purifn., PEGylation, and further polishing of SY161 to yield a formulation that is suitable for use in human clin. trials. Cytoplasmic SY161, expressed in E. coli at > 1 g/L of broth, was recovered by centrifugation and cells were lysed by microfluidizer. The lysate was then oxidatively sulfonated. Most of DNA and ***endotoxin*** were ***removed*** by PEI flocculation; diatomaceous earth filtration was then used to clarify the prepn. All of the product was recovered and significant redn. of ***endotoxin*** and residual DNA (approx. 3 logs) were achieved in the recovery step. The purifn. process was simplified by the addn. of the sulfonate group to the single cysteine (S3C) in the SY161 mol. ***Hydrophobic*** interaction and anion exchange ***chromatog*** . steps increased product purity to > 90%. Cysteine-targeted PEGylation was employed for the mol. modification after lysis of the sulfonate adduct. Further purifn. of PEGylated SY161 using cation exchange to ***remove*** the excess PEG, and anion exchange to polish the product, resulted in a product that meets the safety, purity, and potency requirements for injectable biotherapeutics.

L35 ANSWER 13 OF 17 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:776398 HCAPLUS
DOCUMENT NUMBER: 132:248287
TITLE: Synthesis of labeled analogues and functional study of lipid A, the active principle of bacterial ***endotoxin***
AUTHOR(S): Oikawa, Masato; Liu, Wen-Chi; Furuta, Hiroki; Shintaku, Tetsuya; Sekljic, Harald; Fukuda, Naohiro; Fukase, Yoshiyuki; Suda, Yasuo; Fukase, Koichi; Kusumoto, Shoichi; Kirikae, Teruo
CORPORATE SOURCE: Graduate School of Science, Osaka University, Japan
SOURCE: Tennen Yuki Kagobutsu Toronkai Koen Yoshishu (1999), 41st, 133-138 CODEN: TYKYDS
PUBLISHER: Nippon Kagakkai
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
TI Synthesis of labeled analogues and functional study of lipid A, the active principle of bacterial ***endotoxin***
AB A review and discussion with 8 refs. on the authors' work. To elucidate the mechanism of the biol. events caused by bacterial ***endotoxin*** (lipopolysaccharide, ***LPS***), isotope and fluorescence labelings were effected on lipid A, which is the partial structure of ***LPS*** essential for its bioactivity. The labelings were performed by means of chem. synthesis, and physicochem. as well as biol. functions of the labeled derivs. were evaluated. 6-13C-Labeled derivs. were synthesized of a biosynthetic precursor of lipid A (6-13C-2) and its analog with shorter (C10) acyl chains (6-13C-3). NMR study of the biosynthetic precursor using 13C-labeled and unlabeled specimens enabled us to figure out its supramol. structure formed by self-assembly. By contrast, the short-acyl analog was found to form no aggregate. Mol. modeling revealed the origin of this striking behavioral and conformational difference: unnatural C10 length for the acyl moieties is not sufficient to obtain stabilization by ***hydrophobic*** interaction. Because it has already shown that the short-acyl analog no longer retains the biol. activity of the biosynthetic precursor, it is strongly suggested such a supramol.-forming ability relates with the biol. activity of lipid A analogs. The fluorescence-labeled lipid A analog was next synthesized to obtain a deeper insight on the self-assembly of lipid A. BODIPY was employed as a fluorescence group, and the labeling was effected on the phosphonooxyethyl (PE) analog wherein the chem. labile glycosyl phosphate of lipid A is replaced with the stable 2-(phosphonooxy)ethyl group. The synthesis was achieved using allyl-type groups for the persistent protection of all active functionalities to afford the highly pure final product. The labeled analog was found to retain definite activities of lipid A. Fluorescence spectra indicated the crit. micelle concn. of labeled analog being below 0.46 nM. From these expts. it can be clearly concluded that the labeled lipid A analog forms aggregate around the concn. range where it exhibits the biol. activities. To identify the possible receptor(s) on the competent animal cells, tritium-labeling was effected on the ethylene glycol moiety of the PE analog of lipid A. Careful two-step oxidn. of the allyl group and partition ***chromatog*** . purifn. after final deprotection led to complete ***removal*** of over-oxidized byproducts and eventually enabled us to obtain a highly pure (>98% radiochem. purity) prepn. of 3H-PE analog with high specific radio activity (62 GBq/mmol). Using the 3H-labeled and unlabeled PE analogs, four binding proteins were detected in macrophages from both ***LPS*** -responder C3H/HeN mice and ***LPS*** -hyporesponder C3H/HeJ mice.
IT Proteins, specific or class
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(membrane; synthesis of labeled analogs and functional study of lipid A, active principle of bacterial ***endotoxin***)
IT Adhesion, biological
Gram-negative bacteria
Macrophage
Virulence (microbial)
(synthesis of labeled analogs and functional study of lipid A, active principle of bacterial ***endotoxin***)
IT Lipopolysaccharides
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(synthesis of labeled analogs and functional study of lipid A, active principle of bacterial ***endotoxin***)
IT Lipid A
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(synthesis of labeled analogs and functional study of lipid A, active principle of bacterial ***endotoxin***)

L35 ANSWER 14 OF 17 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:132231 HCAPLUS
DOCUMENT NUMBER: 118:132231
TITLE: Preparation of amino- and hydrophobic group-containing silica gel derivatives as adsorbents of pyrogens
INVENTOR(S): Hidachi, Kiyoshi; Okawa, Kohei; Fujikake, Shiro; Iida, Noboru
PATENT ASSIGNEE(S): Mitsui Toatsu Chemicals, Inc., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 3 pp. CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 04256434	A2	19920911	JP 1991-103228	19910206

AB A silica gel contg. NH2 and ***hydrophobic*** group is prepd. as

adsorbent for pyrogens of injection solns., transfusion solns., dialysis solns., etc. A dispersion of porous globular silica gel with ***chromatog*** . grade in THF was treated with Et2N(CH2)3Si(OEt)3 for 2 h to give an adsorbent. A soln. for renal dialysis contg. 1.4 ng/mL ***endotoxin*** was passed through a column packed with the adsorbent to give an eluate contg. 2.6 pg ***endotoxin*** /mL.

IT Pharmaceutical dosage forms
(pyrogen ***removal*** from, amino- and hydrophobic group-contg. silica gel as adsorbents for)

L35 ANSWER 15 OF 17 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1992:82238 HCAPLUS
DOCUMENT NUMBER: 116:82238
TITLE: Indolyl-3-alkane .alpha.-hydroxylase manufacture with Pseudomonas and its uses
INVENTOR(S): Murtfeldt, Robert; Bream, Allan J.; McCarthy, Kathryn
PATENT ASSIGNEE(S): Automedix Sciences, Inc., USA
SOURCE: PCT Int. Appl., 59 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9118104	A1	19911128	WO 1991-US3630	19910523
W: CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
US 5244807	A	19930914	US 1990-528681	19900523
US 5723325	A	19980303	US 1996-648620	19960516
PRIORITY APPLN. INFO.:			US 1990-528681	19900523
			US 1993-122041	19930914

AB Indolyl-3-alkane .alpha.-hydroxylase (INDH) isoenzymes for use in the ***removal*** of tryptophan from biol. samples (e.g. in extracorporeal circulation) are prepd. free of endotoxins from Pseudomonas XA. The enzyme is produced by stationary phase cultures under O satn. The enzyme was purified from cell paste lysates prepd. with a high pressure homogenizer by ion-exchange, ***hydrophobic*** interaction, and gel-exclusion ***chromatog*** . Two forms of the enzyme were found, both are heterotrimers with subunit mol. wts. of 32,500, 34,500, and 75,000 (INDH-1) and 42,000, 44,000 and 60,000 (INDH-2). Using N-acetyl-L-tryptophanamide as substrate, the enzymes had a specific activity of .apprx.25 units/mg. ***Endotoxin*** contamination was 0.04 ng ***endotoxin*** /enzyme unit. Enzyme immobilized on siloxane-treated silica beads (320 units/56 mL packed silica beads) was used to ***remove*** tryptophan from plasma in a plasmapheresis system. Up to 100% of the tryptophan present in the plasma was ***removed*** from samples; efficiency of ***removal*** depended to some extent upon the starting concn. of tryptophan in the blood. Toxicity studies are also reported.

ST indolyl alkane hydroxylase manuf Pseudomonas; tryptophan ***removal*** blood enzymic extracorporeal

IT Immobilization, biochemical
(of indolylalkane hydroxylase from Pseudomonas, for ***removal*** of tryptophan from blood)

IT Blood
Blood plasma
(tryptophan ***removal*** from, immobilized indolylalkane hydroxylase for)

IT Toxins
RL: BIOL (Biological study)
(endo-, indolylalkane hydroxylase free of, from Pseudomonas, for ***removal*** of tryptophan from blood)

IT Circulation
(extracorporeal, tryptophan ***removal*** in, with immobilized indolylalkane hydroxylase)

IT 63363-76-8D, Indolyl-3-alkane .alpha.-hydroxylase, immobilized
RL: BIOL (Biological study)
(for ***removal*** of tryptophan from blood)

IT 63363-76-8P, Indolyl-3-alkane .alpha.-hydroxylase
RL: BMF (Bioindustrial manufacture); BIOL (Biological study); PREP (Preparation)
(manuf. of, with Pseudomonas XA, for ***removal*** of tryptophan from blood)

IT 73-22-3, Tryptophan, uses
RL: USES (Uses)
(***removal*** from blood of, with indolylalkane hydroxylase from Pseudomonas)

L35 ANSWER 16 OF 17 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1991:435560 HCAPLUS
DOCUMENT NUMBER: 115:35560
TITLE: Chromatographic ***removal*** of ***endotoxin*** from a macromolecular antitumor antibiotic SN-07
AUTHOR(S): Ishida, Seiji; Saeki, Jun; Kawashima, Toshiyuki; Kumazawa, Eitaro; Katoh, Shigeo; Sada, Eizo
CORPORATE SOURCE: Snow Brand Milk Prod. Co. Ltd., Tochigi, 329-05, Japan
SOURCE: Kagaku Kogaku Ronbunshu (1991), 17(3), 589-94
CODEN: KKRBAW; ISSN: 0386-216X
DOCUMENT TYPE: Journal
LANGUAGE: Japanese

TI Chromatographic ***removal*** of ***endotoxin*** from a macromolecular antitumor antibiotic SN-07

AB ***Endotoxin*** was sepd. from a macromol. antibiotic SN-07 by ion-exchange ***chromatog*** . (IEC) and ***hydrophobic*** interaction ***chromatog*** . (HIC). For IEC and HIC, ***endotoxin*** -free columns were prepd. by washing the adsorbent gels with alk.-ethanol followed by repeated wash with buffers of high and low concns. of salt by turns. Using an ***endotoxin*** -free column (Sepabeads FP-DA 13) for IEC, 95.5% of charged ***endotoxin*** was ***removed*** from the SN-07 fractions. For HIC, two types of adsorbent gel, Sepabeads FP-PH 12 and FP-PH 13, were used. FP-PH 13 has a higher ligand concn. than that of FP-PH 12. For both gels the differences in adsorption capacity for ***endotoxin*** at these salt concns. of NaCl and (NH4)2SO4 were slight. In HIC purifn. of SN-07, the ***removal*** of ***endotoxin*** involved in the SN-07 fractions after purifn. by IEC was as high as 92.8% for FP-PH 12 and 95.7% for FP-PH 13. This result corresponded to the absorption capacity of both gels for ***endotoxin***

ST ***endotoxin*** ***removal*** antibiotic SN07 chromatog

IT Toxins
RL: REM (Removal or disposal); PROC (Process)
(endo-, ***removal*** of, from antitumor antibiotic SN-07 by chromatog.)

IT 100753-80-8P, SN 07
RL: PREP (Preparation)
(endotoxins ***removal*** from, by chromatog.)

L35 ANSWER 17 OF 17 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1989:509661 HCAPLUS
DOCUMENT NUMBER: 111:109661
TITLE: Renaturation and purification of biologically active recombinant human macrophage colony-stimulating factor expressed in E. coli
AUTHOR(S): Halenbeck, Robert; Kawasaki, Ernest; Wrin, Joe; Koths, Kirston
CORPORATE SOURCE: Dep. Prot. Chem., Cetus Corp., Emeryville, CA, 94608, USA
SOURCE: Bio/Technology (1989), 7(7), 710-15
CODEN: BTCHDA; ISSN: 0733-222X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Recombinant human macrophage colony-stimulating factor (M-CSF), a disulfide-linked dimeric protein contg. 18 cysteines, was purified in monomeric form from E. coli and renatured (in the presence of glutathione) to generate fully active dimers with an overall yield of 25%. Residual contaminants, including ***endotoxin*** , were ***removed*** from the refolded M-CSF by hydrophobic interaction. The refolded M-CSF

closely
resembles both native and recombinant M-CSF produced by mammalian cells.
IT Toxins
RL: REM (Removal or disposal); PROC (Process)
(endo-, ***removal*** of, from human recombinant macrophage colony-stimulating factor, by ***hydrophobic*** interaction ***chromatog*** .)